Review Article

Multifunctional DNA Nanomaterials for Biomedical Applications

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The rapidly emerging DNA nanotechnology began with pioneer Seeman's hypothesis that DNA not only can carry genetic information but also can be used as molecular organizer to create well-designed and controllable nanomaterials for applications in materials science, nanotechnology, and biology. DNA-based self-assembly represents a versatile system for nanoscale construction due to the well-characterized conformation of DNA and its predictability in the formation of base pairs. The structural features of nucleic acids form the basis of constructing a wide variety of DNA nanoarchitectures with well-defined shapes and sizes, in addition to controllable permeability and flexibility. More importantly, self-assembled DNA nanostructures can be easily functionalized to construct artificial functional systems with nanometer scale precision for multipurposes. Apparently scientists envision artificial DNA-based nanostructures as tool for drug loading and *in vivo* targeted delivery because of their abilities in selective encapsulation and stimuli-triggered release of cargo. Herein, we summarize the strategies of creating multidimensional self-assembled DNA nanoarchitectures and review studies investigating their stability, toxicity, delivery efficiency, loading, and control release of cargos in addition to their site-specific targeting and delivery of drug or cargo molecules to cellular systems.

1. Introduction

Public healthcare is a big issue among the society and has drawn much attention to general public. In general, some organic small-molecules, proteins, and nucleic acids have exhibited their promise as therapeutic agents for biomedical therapy. In the past years, scientists dreamed of improving the delivery efficacy of these target drugs for various biological and biomedical applications. However, problems in terms of solubility, toxicity, cost, and penetration ability need to be solved. They face several transport barriers after they are introduced to human body, before going to their sites of action. For example, first, drug molecules have to be stable in the circulation system, passing through the blood vessel and being recognized by those particular diseased cells. Afterwards, they have to pass through the highly chargeable plasma membrane and/or the nuclear membrane. They also have to withstand the acidic cellular environment. Finally, the multiple drug resistance mechanism also needs to be considered. Thus, it is of great importance developing smart

system which exhibits specific targeting and has high delivery efficacy of active drug molecules.

Scientists envision the rapid development of material sciences offering great advantage for creating smart drug delivery vehicles or carriers. Various drug delivery systems based on different materials have been developed [1]. For example, drugs can be loaded onto the nanoparticles [2] or nanodiamonds [3] for targeted delivery. Active biomolecular drugs can be coordinated to metals inside the carbon nanotube and then released by heating up the nanotubes samples [4]. Another advanced development is to deliver siRNA by PEGylated cyclodextrin molecules [5]. They were released by dissociation of the complexes in lysosome. Particularly, the most commonly used drug delivery system is the polymeric materials [6]. The diblock copolymers tend to form micelle in the presence of drug molecules. Therefore, drug can be easily loaded into the core of micelle [7]. However, being useful drug nanocarriers, it is necessary to consider their toxicity, biocompatibility, and stability in a cellular environment. It is well-known that most of the nanoparticles are toxic; they

may induce cytotoxicity in living systems. Heat triggered-release of drug molecules in a cellular environment is not appreciated because other healthy cells may also be affected. In addition, the efficiency and selectivity of drug loading in polymeric micelles is also highly limited. Therefore, to design new materials as drug carriers, these carriers should have a capability of drug incorporation and controlled release in a highly effective way. They should also be highly stable and biocompatible in a specific cellular environment. It is also necessary for them to target particular areas and carry multifunction in order to enhance the delivery efficiency.

Indeed, developing novel biocompatible and multifunctional nanocarriers remains a key challenge for targeted drug delivery. The rapidly emerging DNA nanotechnology began with pioneer Seeman's hypothesis that DNA not only can carry genetic information but also can be used as molecular organizer to create well-designed and controllable nanomaterials for applications in materials science, nanotechnology, and biology [8, 9]. As DNA has a simple and robust molecular recognition rule of adenine to thymine (A-T) and guanine to cytosine (G-C) pairings, two complementary single-stranded DNA hybridize to form a double helix with predictable and programmable interactions. The structural features of nucleic acids form the basis of constructing a wide variety of well-ordered DNA nanoarchitectures with well-defined shapes and sizes, in addition to controllable permeability and flexibility [10, 11]. This DNA nanotechnology offers new opportunities for the construction of complex DNA structures in different dimensions. More importantly, self-assembled DNA nanostructures can be easily functionalized to construct artificial functional systems for multipurposes. Apparently scientists envision artificial DNA-based nanostructures as tools for drug loading and *in vivo* targeted delivery because of their potential of selective encapsulation and stimuli-triggered release of cargo.

In this review article, we concentrate on a new-comer of drug delivery carriers based on self-assembled DNA nanostructures. We will demonstrate the power and promise of DNA as a scaffold to create DNA nanostructures with precise geometry and versatile functionality. Their structural stability in physiological conditions and internalization will be briefly described. Different cargo loading mechanisms and their control release via external stimuli will be summarized in detail. As a new-comer in drug delivery system, studies of intracellular behaviors/functions of drug loaded DNA nanocarriers and their interactions in specific intracellular compartments *in vitro* or *in vivo* will also be discussed. Some concluding remarks will try to ascertain what the next challenges and outlook of this exciting research area could be.

2. DNA Nanotechnology

To begin with, we first briefly introduce the history and the most updated status of DNA nanotechnology. The innovation of the field of DNA nanotechnology was first demonstrated by Seeman in the early 1980s [12]. Taking advantage of self-recognition property of DNA, his group designed and constructed modified Holliday junctions to convert one-dimensional DNA strands into branched DNA tiles with

sticky ends at the edges (Figure 1(a)). These short single-stranded units provide toeholds for further assembly of 2D-structures [13]. Since then, the structural role of DNA is widely well-recognized and extensively explored. However, these assembly approaches did not offer rigid junctions with well-defined angles and geometry of the final structures. To overcome these drawbacks, researchers started to develop advanced rigid junctions including multicrossover [14–16], cross-shaped tile with arms [17], DNA tensegrity triangle [18], and parallelogram DNA tile (Figure 1(b)) [19]. With such unprecedented talent to construct DNA-based architectures, highly ordered 2D-DNA surfaces with programmable arrangement and a large variety of three-dimensional polyhedral structures were successfully assembled via sticky-end cohesion among those building blocks [20–22]. Nevertheless, these tile-based assemblies have certain limitations. For example, it is difficult to control the size of resulting structures. An exact stoichiometric and a high purity control of individual DNA fragments are still problematic for the assembly of large and complex nanostructures.

Another creation in DNA nanotechnology was made by Rothemund in 2006 [23]. He invented scaffolded DNA origami which successfully offered high complexity and versatility in DNA assembly. In DNA origami, a long piece of single-stranded DNA from the M13 circular bacteriophage genome is folded with itself into a desired pattern with the assistance of short staple strands (Figure 1(c)) [24, 25]. Typical examples consist of nonperiodic 2D-structures, such as a map of the Americas, stars, smiley faces, and other deliberately well-designed patterns [26, 27]. In this approach, the relative stoichiometric ratio on different staple strands to a single DNA scaffold is not highly restricted. More importantly, DNA origami is a versatile and simple one-pot assembly to generate nanostructures with complex shapes of predefined dimensions as compared to the conventional crossover approach [28, 29]. In an advanced development, Kostiaainen's group has recently demonstrated the optical control of the DNA origami formation and release [30]. Although DNA was used as the only component to guide the DNA assembly in tile-based assembly or DNA origami, this resulted in fully double-stranded and DNA-dense structures.

An alternative approach to building DNA nanostructure is to bring together the programmability of DNA with functional and structural diversities offered by supramolecular chemistry [31]. This new emerging area in DNA nanotechnology involves the insertion of synthetic molecules into DNA strand to alter its hybridization and control the assembly outcome (Figure 1(d)). By conjugating synthetic molecules at the insertion points of a DNA strand, typical linear DNA duplexes can be oriented and hybridized relative to one another in a controlled manner. This supramolecular DNA assembly combines the diverse structural features of molecules and their functionalities such as luminescence, redox, magnetic, and catalytic properties to generate discrete well-defined structures.

Taking advantages of synthetic molecules as rigid junctions, this can reduce the amount of DNA strands needed for the structural definition as compared to the previous two methods. For example, Sleiman's group have successfully

FIGURE 1: Examples of self-assembled DNA nanostructures: (a) A lattice is formed by hybridization of the sticky ends of a Holliday junction; (b) multistranded junction structures and crossover structures including double-crossover structure; cross-shaped tile with four arms; DNA tensegrity triangle and parallelogram DNA tile; (c) the principle of DNA origami and the design of 2D origami formed smiling face and star; (d) sequential self-assembly of hexagonal-shaped DNA nanostructure via supramolecular DNA assembly.

developed DNA-conjugated *m*-terphenyl-based organic vertices for modular construction of cyclic polygons, a library of DNA polyhedral structures and nanotubes with good control over their geometry [32], dimension [33], and flexibility [34]. Besides the organic insertions, other important self-assembly strategies take advantages of transition metal-, ligand-, lipid- and block copolymer-based environments [35–37].

3. Stability of Self-Assembled DNA Nanostructures

Among various DNA assemblies, three-dimensional DNA nanostructures hold promise to be the universal nanocarriers

for smart and targeted drug delivery. In contrast to 1D or 2D DNA structures, the power of self-assembled 3D DNA nanostructures lies in their excellent stability and biocompatibility, high drug loading capability, and passive delivery into live cells. They also possess fine control over geometry, precise and monodisperse dimensions, positioning of guest molecules, stimuli-responsive switching of structure, and triggered-release of cargos. Typical examples of drug delivery systems based on 3D DNA nanostructures [38, 39] include tetrahedron, icosahedron, hexagonal barrel, nanotube, DNA origami box [40], nanorobot, and nanocage.

To be employable as a drug carrier system in mammals, DNA nanostructures must meet several important criteria:

TABLE 1: Stability of different DNA nanostructures.

	Linear dsDNA	CpGbearing DNA tetrahedral nanostructure	A 3D multilayer rectangular parallelepiped structure

Description of the structure	Normal linear DNA strand with DdeI restriction site	It is made up of four 55-mer strands extended with the CpG sequence and a 7-mer oligothymine spacer	A 3D multilayer rectangular parallelepiped structure (8 helix $\times$ 8 helix square lattice with dimensions of 16 nm $\times$ 16 nm $\times$ 30 nm)
Incubation temperature	/	37°C	25°C
Medium	10% FBS	50% non-heat-inactivated fetal bovine serum (FBS)	Cell lysate
Decay time	Decay after 0.8 h	Start decaying after 4 h, but still not completely decayed after 24 h	Still remains stable after 12 h
Citation	[41]	[43]	[44]

(1) they have to be stable and intact in both extracellular and intracellular environments, particularly stable long enough in the cytoplasm of cells to perform their predefined tasks; (2) they should not have toxic effect in mammals; and (3) the cellular immune system in mammals should tolerate the nanometer-scale DNA nanocarrier systems. Thus far, several research groups have put efforts on the stability studies of DNA constructs. Bermudez's group indicated that oligonucleotide-based tetrahedral made from branch junctions exhibit a strong resistance to enzymatic digestion compared to the linear counterparts in terms of their decay time constants (Table 1) [41]. The reason behind this would highly be due to the steric hindrance effect. Since the endonucleases initially bind to the DNA nonspecifically with a low affinity and then follow by diffusion along the strands. The steric hindrance introduced by three-dimensional tetrahedron would reduce the effective binding of enzymes to DNA and then inhibit DNA cleavage, no matter if the enzyme acts specifically or nonspecifically. Furthermore, shorter sequence or smaller size of DNA complex can enhance the resistance towards various nucleases as they are more difficult to bend and possibly have higher steric hindrance for the action of the enzymes. Walsh and coworkers have demonstrated the first example of 3D DNA nanostructure which can enter live mammalian cells effectively with or without the help of a transfection reagent [42]. They stay intact for up to 48 h in cytoplasm. In a recent study by Li et al., they have modified the tetrahedral with CpG oligonucleotides which have been confirmed to be taken up by macrophage RAW264.7 cells effectively (Table 1) [43].

Regarding scaffold DNA origami, Mei and coworkers demonstrated that different shapes of DNA origami nanostructures are stable and remain intact for 12 h after exposing

to cell lysates of various cell lines and can be easily purified from lysate mixtures, in contrast to single-stranded or duplex DNA (Table 1) [44]. They are not accessible to various DNAses due to negatively charged, large, and rigid origami structures. Their superior structural integrity and versatile functionality are highly preserved in relation to conventional oligonucleotides, validating their use for various biological applications. Subsequently, a further study carried out by Dietz' group tested the enzymatic digestion of DNA origami structures [45]. They are fully exposed to a large variety of endonucleases, including DNase, T7 exonuclease, T7 endonuclease, MseI restriction endonuclease, Lambda exonuclease, and *Escherichia coli* exonuclease. These results indicated that they are highly stable at 37°C towards degradation as compared to duplex plasmid oligonucleotides. More recently, Schüller and his coworkers reported that CpG oligonucleotides-decorated DNA origami tubes amplify a strong immune response, which are completely dependent on TLR9 stimulation in mammalian spleen cell [46].

To further optimize DNA structures in regard to enzymatic digestion resistance, Sleiman's group has modified 3D DNA nanostructures using a number of chemical strategies. They found that simple chemical modification to both ends of DNA oligos with hexanediol and hexaethylene glycol in self-assembled DNA prismatic cage or site-specific hybridization of DNA-block copolymer chains to 3D DNA scaffold would dramatically enhance its nuclease resistance under fetal bovine serum condition (Table 2) [47]. These studies could provide guidelines for decoration of DNA nanostructures with simple chemistry modification and allow imparting momentous stabilization towards nuclease degradation. Meanwhile, the same group also demonstrated that creation of DNA nanotubes with a template generated by rolling circle

TABLE 2: Stability of modified DNA nanostructures generated from supramolecular DNA assembly.

	Triangular prism1	Triangular prism2	Nanotube	RCA-nanotube

Description of the structure	Made up of three 96-mer strands with 20 bp edges	Made up of three 96-mer hexaethylene glycol (HEG) modified strands with 20 bp edges	Triangular prism built up by small unit with short linking DNA strand	Connect small triangular prism units with RCA synthesized DNA strand
Incubation temperature	37°C	37°C	/	/
medium	10% FBS	10% FBS	10% FBS	10% FBS
Decay time	18 h	62 h	1.1 h	3.5 h
Citation	[47]	[47]	[32, 48]	[48]

amplification (RCA) results in increased stability towards nuclease degradation as compared to their previous nanotube design (Table 2) [48]. On the other hand, the high density of DNA and aspect ratio of the RCA-templated DNA nanotubes offer a greater cell penetration ability over normal DNA oligos. Such enhanced cellular stability and nuclease susceptibility are the key requirements for DNA nanostructures to act as delivery carriers or vehicles.

To modulate the stability and uptake profile of self-assembled DNA nanocube, Sleiman's group recently decorated their DNA cubes with hydrophobic (dodecane alkyl, C12) or hydrophilic (hexaethylene glycol, HEG) dendritic DNA chains [49] or block copolymers on the edges [50]. They found that all of the integrating dendritic DNA chains were facing outward, as confirmed by a larger hydrodynamic radius from dynamic light scattering (DLS) study and lower mobility band on gel electrophoresis. In addition, this chemical modification would allow enhancing their cellular stability with a longer half-life as compared to the blunt-ended nanocubes. More importantly, they found that the hydrophobic chains on the cube favor rapid and increased cellular uptake while the hydrophilic chains favor slow and continuous internalization.

4. Cargo Loading and Cellular Delivery

In response to the well-defined and highly programmable properties of DNA-based nanostructures, precise control

of positioning of cargo molecules in DNA nano-objects is highly possible. This valuable property is hardly attainable with inorganic or organic nanomaterials. In general, cargo molecules can be loaded via different strategies such as covalent linkage, nucleic acid base-pairing, biotin-avidin interaction, intercalation, aptamer-target interaction, DNA-protein interaction, and encapsulation.

4.1. Covalent Linkage. To deliver the cargo with the aid of DNA nanostructures, some of the cargos can form covalent bonds with DNA strand in the presence of some molecular linkers. Sleiman's and Mao's groups have shown that self-assembled DNA nanotubes act as carriers to deliver cyanine fluorescent dyes into human cancer cells [48, 51]. In Mao study, Cy3 is covalently conjugated to some of the nucleic acid strands at their 5' ends via a well-established N-hydroxysuccinimide (NHS) chemistry. Cy3-functionalized DNA nanotubes were formed by mixing DNA strands with and without Cy3 molecules after a heat-cool cycle. Fluorescent dyes are the most commonly used model cargo for targeted delivery, because they can easily be visualized and traced under various fluorescence microscopes. Taking advantage of automatic solid-phase DNA synthesis, a wide range of fluorescent probes can be readily coupled and labeled on DNA stands. With/without the help of targeting moieties, these structures could be internalized by tumor cells. The fluorescence of the dyes could be localized with fluorescent microscopy, confirming the presence of DNA

FIGURE 2: (a) Different kinds of antibodies have been tagged on the nanorobot and it can identify different antigens on different cells. (b) The complementary strand is incorporated inside the cavity of the nanocage for encapsulation of gold.

nanoassemblies in cells. Moreover, we are able to precisely control the numbers and positions of these fluorescent cargos such that multiple fluorophores can be labeled on a single DNA nanostructure [42, 52].

4.2. Nucleic Acid Base-Pairing. Hybridization of cargo-consisting of single-stranded nucleic acids offers an alternative strategy for site-specific loading of cargos. The nanorobots produced by Church's group have been chemically modified via covalent attachment of 15-base ssDNA linkers as loading sites to the 5' ends of payloads (Figure 2(a)) [53]. In this structure, twelve loading sites were generated. Subsequently, two types of cargo linkers have been prepared in the following ways: gold nanoparticles covalently conjugated to 5'-thiol-functionalized DNA linkers, and Fab' antibodies were covalently conjugated to 5'-amine-functionalized DNA linkers. Mixing the cargo linkers and the nanorobot in aqueous buffer, the staple strands with 3' extensions localized at the loading sites hybridized with the complementary sequences of cargo linkers. Eventually, two different types of payload molecules are loaded successfully per robot. In their design, different Fab' antibody fragments were bounded covalently to the amine-modified linkers. They found that the antibodies were recognized by certain cell-surface receptors and thus inhibited the growth of the targeted cells. In addition, generality of using these barrel structures as carrier is highly possible because a decrease in T cell activation activity that was observed when Fab fragments targeted to human CD3 and flagellin were loaded on these hexagonal barrel structures.

Mao's group has designed a series of symmetric DNA polyhedral structures consisting of two unpaired, ss DNA tails sticking out on each edge (Figure 2(b)) [54]. When mixing the gold nanoparticles functionalized with DNA

strands (DNA-AuNPs), the DNA-AuNPs are swallowed into the polyhedral structures governed by nucleic acid base pairing between the ssDNA tail on the DNA polyhedral structures and the complementary DNA strands immobilized on AuNPs. The size and number of guest molecules trapped by these DNA polyhedra highly depend on their internal volumes.

An alternative molecular cargo drawing attention is RNA interference (RNAi). It becomes a powerful therapeutic agent to knock down the gene expression, inducing gene silencing. Small interfering RNAs (siRNAs) are chemically synthesized nucleic acids with specific sequences which bind to their complementary mRNA molecules and thus inhibit the corresponding protein synthesis, leading to targeted gene knockdown. By choosing the appropriate siRNA sequence, it is possible to restrain the target gene expression which causes diseases. Anderson and coworkers have successfully developed a new siRNA delivery system by incorporating six double-stranded siRNAs to tetrahedral DNA assemblies. The single-stranded overhangs on DNA strands allow the specific hybridization of complementary siRNA sequences and cancer targeting ligands with better control over their spatial orientation, locations, and density. These nanostructures have been applied in female BALB/c nude mice model bearing Luc-KB tumor. They found that RNA-modified DNA nanostructures are able to knock down the luciferase levels in terms of the protein and mRNA levels, leading to target genes silencing in tumor cells. Importantly, they exhibit a longer blood circulation time than the parent siRNAs do. This work highlights the significance of DNA nanostructures to improve the biostability of tethered RNA strand, thus greatly enhancing the RNAi efficacy in nanomedicine [55].

Recently, Sleiman's group has integrated the firefly Luciferase antisense strands into the DNA triangular prism.

FIGURE 3: A diagram showing the effect on luminescence of bear PS and PS-integrated DNA triangular prism.

They demonstrated that DNA prisms composed of antisense strands can significantly induce gene knockdown in HeLa cells without being influenced by conjugating small fluorescent probes within the structure and by serum conditions. The RNA-modified DNA prisms maintain gene silencing up to 72 h and are still significantly powerful at an initial stage of gene knockdown after they are removed (Figure 3) [56].

In addition, unmethylated cytosino-phosphate-guanine (CpG) oligonucleotides are classified as therapeutic nucleic acids, with a strong immunostimulatory effect [26]. The CpG sequences are commonly present in bacterial and natural viral DNA for immune response, invading pathogens in a host [57, 58]. Interestingly, it is found that CpG oligonucleotides can effectively be recognized by endosomal Toll-like receptor 9 (TLR9) and further induce conformational changes simultaneously [59, 60]. This process ultimately triggers a signaling cascade which leads to the powerful immunostimulatory properties of CpG oligonucleotides. They can be highly used for the immunotherapy of cancer and infectious diseases [61, 62]. However, natural CpG oligonucleotides are easily digested by nucleases in biological systems and difficult to pass through the plasma membrane, entering cell and reaching their target sites. In this regard, it is necessary to develop a nanocarrier with low cytotoxicity and high delivery efficacy for clinical uses of CpG. Given that self-assembled well-defined DNA nanostructures are rigid and insensitive to nuclease digestion, several research groups have appended CpG motifs to multidimensional DNA structures in order to evaluate their uptake efficiency, stability, and immunoregulatory effects.

Nishikawa et al. designed and assembled a Y-shaped DNA unit from three single-stranded DNAs. Interestingly, CpG sequences have been introduced to these strands [63]. They found that Y-shaped DNA units induced a great immune response from RAW264.7 cells compared to ss- or ds-DNAs in terms of producing a higher amount of proinflammatory cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6). These units also exhibited higher uptake efficiency in macrophage-like cells than natural ds DNAs. Subsequently, the same group further applied this Y-shaped DNA unit to assemble dendrite-like nanostructures. Surprisingly, they demonstrated even a stronger immune

response by inducing a larger amount of proinflammatory cytokines from RAW267.4 cells than the monomer Y-shaped DNA units do [64]. Recently, Nishikawa's group developed a series of nanometer-scale polypodna consisting of CpG motifs and examined their structural and immunological properties. Particularly for hexa- and octapodna; they could highly induce the secretion of TNF- α and IL-6 from RAW264.7 cells. Interestingly, large numbers of pod could increase the cellular uptake but also reduce their stability in serum condition. This enhanced stimulatory activity suggests the importance of the stereochemical property of self-assembled DNA nanostructures.

Recently, Li and coworkers have successfully developed a DNA tetrahedron as a CpG nanocarrier [43]. These nanometer-scale 3D structures are structurally rigid, mechanically stable, and nontoxic. They are also highly stable in serum condition and resistance to nuclease digestion in live cultured cells for few hours. As compared to ssDNA, the CpG-functionalized DNA tetrahedral structures can enter RAW264.7 cells efficiently. Importantly, this tetrahedron acts as a carrier to deliver the CpG therapeutic nucleic acids to acquire immune response. The amount of certain cytokines including TNF- α , IL-6 and IL-12 stimulated by them were remarkably increased than those by ss CpG nucleic acid strand. In addition, DNA tetrahedral could load more than one CpG, resulting in even higher stimulatory activity. In such case, the positions of CpG loading can be used to monitor the dose of drug molecule precisely. Additionally, several groups have successfully developed a large variety of origami structures for large amount of CpG loading, leading to a strong immune cell activation in freshly isolated spleen cells or in RAW 264.7 cells by cytokine production in a high level (Figure 4) [46, 65]. In overall, it is highly suggested that various geometries of DNA nanoobjects have shown advantages of cellular delivery and immunostimulatory activity of CpG in macrophage-like cells, making DNA nanostructures promising immunotherapeutic carriers.

4.3. Biotin-Streptavidin Interaction. Biotin, also called vitamin H, is a small molecule and exhibits a strong binding affinity to biotin-binding proteins such as avidin or streptavidin. The high affinity of the biotin-streptavidin interaction not only offers useful bioanalytical advantages [66], but also

FIGURE 4: A diagram showing how DNA-tubes CpG go into the cell and functionalize.

FIGURE 5: A DNA origami designed for doxorubicin transportation.

makes this system to be an attractive model for site-specific loading or positioning of guest molecules in highly ordered DNA assemblies [67, 68]. Recently, Gothelf and coworkers have demonstrated a chemical modification of nucleic acid strands with biotin allowing for streptavidin binding at precise positions in a well-defined self-assembled DNA origami scaffold. In this study, biotin-tethered functional groups including an alkyne, an amine, and an azide reacted with their corresponding reactive groups via either a Huisgen-Sharpless-Medal copper(I) catalyzed click chemistry or N-hydroxysuccinimide chemistry. The results of high yield, selective cleavage, and bond formation in this study offer the potential of applying such interaction for site-selective uptake and triggered release of cargos in a control manner [69].

4.4. Intercalation. In DNA chemistry, intercalation is a reversible insertion of a guest molecule into double helix of DNA strands. The small molecules can interact with nucleobases and disturb the π - π stacking of between double-stranded DNA (dsDNA). Doxorubicin is one of the most common drugs that can be trapped by DNA nanostructures. It can intercalate in G-C base pair of DNA strand. It is small and can be trapped by DNA nanomaterials easily [70–72]. Many newly developed DNA nanocarriers have been tested due to its simplicity [73, 74]. There is another example of

doxorubicin carried by DNA origami which can circumvent drug resistance. It enters and localizes in resistance human breast cancer cell (res-MCF-7) while the free doxorubicin cannot enter. The DNA origami increases pH of lysosome in resistant cancer cells, followed by redistribution of drug. This would allow them to go to their target site (Figure 5) [73]. Zhao and his colleagues have also developed a DNA origami tube for transporting doxorubicin. By optimizing the design of nanostructures, encapsulation efficiency and the release rate of the drug can be adjusted [74].

Shen et al. and Zhu et al. also reported the delivery of DNA-based structures to cells in the presence of intercalated dyes including SYBR Green and carbazole-based biscyanine as fluorescent cargo [75, 76]. These dyes can specifically bind to and intercalate with DNA duplex, giving out strong fluorescence. Subsequently, the intercalated dyes are completely released and a decrease in fluorescence is observed once DNA structures are disrupted by some reasons. Importantly, they realized that the enzymatic degradation of these assemblies lasted for at least few hours in cellular environment, resulting in sustainable release of cargo molecules.

4.5. Aptamer-Target Interaction. Aptamers are either ssDNA or ssRNA molecule that can selectively bind to certain targets such as proteins and peptides, with high affinity and

FIGURE 6: Thrombin binding aptamer is introduced into the design of the DNA origami for tagging thrombin.

specificity. These molecules can be presented in a large variety of shapes including helices and single-stranded nucleic acid loops due to their intrinsic propensity and versatility to diverse targets. They can link to various proteins as well as other nucleic acids, small organic compounds, and even entire organisms [77, 78]. Yan's Group has demonstrated the first example of selective DNA aptamer binding as a powerful platform for positioning of proteins in periodic locations of self-assembled DNA arrays (Figure 6) [79]. In these studies, thrombin binding aptamer (TBA) is chosen, which is a well-known 15-base nucleic acid aptamer consisting of specific sequence of d(GGTTGGTGTGGTTGG) [80]. They found that DNA-based array constructed with this TBA can fold into a unimolecular guanine quadruplex and then selectively bind to a protein called thrombin, with nanomolar affinity. This aptamer-target interaction mechanism would provide an alternative choice for cargo uptake with a larger flexibility and simplicity. Only aptamer sequence is required to be implemented in the design of DNA nanocarrier.

In general, aptamers are usually selected from a pool of large random sequences. Because of their high specificity and ease of synthesis, they have been widely used for biosensing and diagnostic applications [81]. More recently, aptamers have become therapeutic candidate as biomedical drugs [82, 83]. Common used human α -thrombin aptamer, which has two binding sites, can be readily loaded on self-assembled DNA structures with appropriate design [84, 85]. Fan's group designed a dynamic DNA tetrahedral nanostructure with an anti-ATP aptamer embedded in one of the edges [60]. This nanostructure could go into cells and monitor the level of ATP via the ATP-induced aptamer conformational change that alters the FRET efficiency of a pair of fluorophores (Cy3 and Cy5) labeled on the structure.

The optical activity of DNA strand used to construct DNA nanomolecules would also affect the structure of nanomaterials. L-DNA and D-DNA has common structure and liability but once the nanostructure is attached to aptamer, mismatching in nanocage made by D-DNA may occur. L-DNA is a better choice for construction because the structure of cage with aptamer is unchanged [86].

4.6. DNA-Protein Interaction. In a cellular environment, there are many different kinds of proteins while some of them can interact with DNA for various cellular reactions. Transcription factor is one of the examples. It has binding site which can interact with DNA sequence. Kapanidis's group

has demonstrated selective trapping of transcription factor (TF) in DNA cage (Figure 7) [87]. Transcription factor is a DNA binding protein which is important in gene regulation. TF catabolite activator protein (CAP) is used as cargo in this experiment. The 22 base pair DNA recognition site is integrated in the DNA tetrahedron. With the presence of cyclic adenosine protein (cAMP), the allosteric effector of protein increases the binding affinity of CAP towards the binding recognition sequence. These results suggested that protein would still be trapped inside the cage even it is already formed, unlike other passive encapsulation methods. The CAP can be released by degradation of cages in presence of DNA nuclease I.

Liu and his coworkers reported a DNA-based delivery system for synthetic vaccines [88]. In their design, biotinylated DNA tetrahedron was used as carrier to deliver antigen streptavidin (STV) into mice with the aid of biotin-STV interaction. Interestingly, the antigen-modified DNA tetrahedron complexes could stimulate strong and continuous antibody responses against the antigen in comparison with antigen itself. On the other hand, unmodified DNA nanostructures did not induce any response. These results indicated the promise of the use of self-assembled DNA nanostructures as a delivery and generic platform for rational design and construction of vaccines.

4.7. Encapsulation. In addition to specific binding interactions between cargos and carriers, payloads can also be directly loaded into container-like DNA nanostructures via passive encapsulation. Recently, Sleiman's group demonstrated the ability of a 3D DNA-based nanoobject to passively encapsulate certain sizes of cargos [89]. DNA nanotubes of longitudinal variation structure have been created in which they can encapsulate gold nanoparticles of specific sizes to form nanoparticle "pea-pod" lines. It is of note that the "sieving" ability is very important, only specific nanoparticle sizes that match the size of the capsules along the nanotubes could be encapsulated, and the process is highly selective. This approach allows controlling of the positioning and loading of a wide range of sizes of guest molecules in a precise way by designing the dimensions of cavities inside the DNA nanoobjects.

Sequentially, Krishnan's group further applied this strategy for the encapsulation of a fluorescent biopolymer, for example, FITC-dextran, in a synthetic icosahedral DNA-based container. Without molecular recognition between

FIGURE 7: The figure is showing that the conformation of bound and unbound CAP integrated in DNA tetrahedron.

the host and guest, cargo molecules are passively loaded to the 3D container during joining the two halves of icosahedron in PBS buffer (Figure 8) [90]. They have reported the delivery of DNA icosahedral encapsulated fluorescent dextran (FD) specifically in *cellulo*, *Drosophila* hemocytes and in *C. elegans* via anionic ligand-binding receptor (ALBR) pathway. The FD cargo is a complex, branched polysaccharide composed of around 10 kDa, 5.2 nm in sizes. It is found that the functionality of the encapsulated FITC-FD in living worms is preserved and the spatially mapping of pH changes during maturation of the endosomes in coelomocytes.

5. Controlled Releases of Cargo Molecules

To act as a nanocarrier for drug delivery, control release of cargo is another significant issue needed to be considered carefully. In the following section, different approaches will be explained and discussed in detail.

5.1. A DNA Strand Displacement. The cargo trapped in DNA nanotube from Sleiman's group is released by strand displacement (Figure 9(a)) [89]. The nanotube is partially hybridized to one strand and gives some tails. Introducing the completely complementary DNA to the tails, the rigidity of the cavity capping gold released. Sleiman has demonstrated selective release of cargo molecules in response to a specific external DNA strand. They have designed and assembled 3D DNA nanotubes with encapsulated gold nanoparticle as well as some modified linking strands consisting of an eight-base overhang protruded from each of their large capsules. After a fully complementary eraser DNA strand is added to these self-assembled nanoobjects, the closing linking strands are erased and hybridized and form a double helix with the complementary eraser DNA strand. The fully doubled-stranded DNA nanotubes become partially single-stranded, so that the encapsulated cargos are released simultaneously.

This release process is highly selective and fast. It is just like unzipping the clothes. As the cavity is more flexible without the rigidified strands, the nanogold can be leaked out easily.

The same group has also applied the same strand displacement technique to release the guest molecules such as the block copolymer micelles loaded on the RCA-nanotubes (Figure 9(b)) [37], and the Nile red or 1,6-diphenyl-1,3,5-hexatriene (DPH) loaded on dendritic alkyl chains-modified DNA cages [91].

Goodman et al. has reported the operation of reconfigurable, braced 3D DNA nanostructure whose structure switches precisely and reversibly in response to specific molecular inputs [92]. Four DNA strands are mixed in solution to form a tetrahedron which consists of a hairpin loop on one edge. This edge can be expanded by adding a fuel DNA strand that is fully complementary to the hairpin region. On the other side, the edge can be contracted by adding the eraser DNA strand which displaces the fuel strand via hybridization of its single-stranded overhang first.

5.2. Addition of Small Molecules. To carefully realize the potential of these 3D DNA nanostructures as nanocarriers, the development of spatiotemporal release of the trapped cargo is of great importance. Recently, Krishnan's group has successfully demonstrated the precise control over the opening of a 3D DNA icosahedron loaded with molecular cargo in response to an external small molecule, called cyclic-di-GMP (cdGMP) (Figure 10(a)) [93]. Generally speaking, cdGMP existed as a second messenger in most bacteria for regulation of various biological processes. In their design, cdGMP aptamers are chosen and have been introduced to the icosahedral design. Upon binding to cdGMP ligands, the aptamer undergoes a conformational change by strand displacement and then dissociate the polyhedral structures into two halves. Simultaneously, the encapsulated fluorescent dextrans are completely released. Therefore, we strongly

FIGURE 8: Cargo molecules are passively loaded onto 3D DNA-based container after joining the two halves of icosahedron in PBS buffer.

FIGURE 9: A DNA nanotube for gold releasing by strand displacement (a) and demonstration of PEG releasing in RCA-DNA nanotube by strand displacement (b).

envision artificial DNA-based nanostructures as nanotool for drug loading and targeted delivery because of their ability for selective encapsulation and stimuli-triggered release of cargo.

5.3. pH Adjustment. pH adjustment is also a possible stimulant for the structural change of DNA nanostructures. The key element of this structural switching mechanism is i-motif switching. It makes use of the properties of Watson-Crick base-pairing and Hoogsteen hydrogen bonding. In an acidic environment, C is partially protonated as C⁺ which can bind with a G-C nucleobase pairs through Hoogsteen H-bonding in order to generate C+G-C triplets. However, C⁺ loses one electron and turns back to C under neutral environment, discarding the Hoogsteen H-bonding and C+G-C triplets simultaneously. Liu et al. reported the first pH responsive DNA tetrahedron in terms of their reversible assembly and disassembly in response to solution pH changes (Figure 10(b)) [94]. In the current design, three-point-star DNA motif can associate with one another to form a DNA tetrahedron in acidic environment (pH at 5) through DNA triplex formation of cytosine-modified sticky ends. While under neutral pH environment, the tetrahedron dissociates into its building blocks immediately. The design can be improved for drug delivery by adjusting pH value towards the formation of DNA tetrahedral. We strongly believe that

such pH-responsive behavior in self-assembled DNA nanostructures will be important for potential applications such as controlled/targeted drug release in specific cellular environments. The same group also developed a pH biosensor based on DNA nanomachine which is triggered by protons to map temporal and spatial pH changes in a cellular system via similar structural switching mechanism [95].

5.4. Photo Irradiation. Compared with the above input signals, photon is an ideal external source for precise control of photo-manipulation of DNA nanoobject. By using light, DNA nanoobjects can be remotely controlled, offering a novel avenue in nanomedicine and drug delivery. Generally speaking, photo irradiation is a clean switching mechanism. NO waste is generated as only light was used to drive the entire process. It offers capability to precise control light irradiation in both temporal and spatial fashions. More importantly, it would not damage the samples as photo irradiation is noninvasive and noncontact source of stimulus. Recently, azobenzene has been confirmed to be a photo-responsive molecule that can be conjugated to nucleic acid strands for the regulation of hybridization-dehybridization process [96, 97]. It exhibited reversible stereoisomerization property. It switches from the *trans* to *cis* conformation when excited at 330–380 nm wavelength of light. On the other side, it reversibly switches from *cis* to *trans* under excitation of

FIGURE 10: (a) By binding the cdGMP to aptamer integrated in DNA icosahedron, nanocage can be opened for molecule releasing. (b) It makes use of the *Watson-Crick base pair* and *Hoogsteen base pair* properties to construct a DNA tetrahedron which can form in low pH and decompose in high pH conditions.

light with wavelength above 400 nm. This intrinsic property of azobenzene allows the photo-manipulation of DNA nanostructures in a precise and control manner. On the basis of this technique, Liang et al. designed photon-fuelled molecular DNA tweezers consisted of photoresponsive azobenzene-modified DNA strand. Photo-induced opening and closing of the tweezers is governed by the irradiation wavelength (Figure 11(a)) [98]. Subsequently, the same group has successfully designed and constructed a supra-photoswitch consisting of alternating natural nucleobase pairs and azobenzene moieties in the form of (AAB) $_n$, where A and B represent the natural nucleotides and the azobenzene, respectively [99]. They found that the stability of the azobenzene modified DNA duplex is more stable than the neutral one. This property is useful in implementing in different DNA nanocarriers. Kang et al. designed and constructed photoswitchable single-molecular DNA motor with tethered azobenzene moiety [100]. This nanomotor is driven by photo irradiation between UV light and visible light without any additional DNA strand as external fuel.

Recently, highly complex DNA nanostructures incorporated with photo-responsive molecule have been successfully designed and generated. Zou and his coworkers constructed DNA nanoscissors composed of two hairpin structures H1 and H2. In this study, a DNAzyme is used as an example system for DNA cleavage (Figure 11(b)) [101]. Particularly, H2 is a complementary azobenzene-functionalized sequence at the 5-end of DNAzyme. Under visible light irradiation, the two hairpins preserve their hairpin structures as duplexes, blocking the substrate binding and closing down DNA cleavage activity. This is in a closed state of DNA nanoscissors. While under UV light irradiation, H2 is able to be opened due to structural isomerization of azobenzene from its planar to nonplanar conformations, prohibiting duplex formation at H2 and then allowing intermolecular hybridization between DNAzyme and the substrate, thus activating the enzymatic

activity. This is in an open state of DNA nanoscissors. They found that the ON and OFF states of nanoscissors lead to a remarkable change in substrate binding affinity and an obvious difference in the activity of DNA cleavage.

Yang and his colleagues have successfully demonstrated the reversible assembly and disassembly of DNA-based structures by introducing azobenzene-modified DNA strands into hexagonal DNA origami units [102]. A number of nanometer-sized hexagonal DNA origami structures functionalized with photo-responsive oligonucleotides have been generated. They can be assembled into a large variety of 2D regular or irregular nanostructures under visible irradiation. On the other hand, DNA hexagonal origami would obtain the *cis*-conformation under UV light irradiation such that they cannot hybridize together due to steric hindrance effects. By altering the numbers and positions of azobenzene-modified oligonucleotides in the hexagonal shaped DNA origami scaffolds, they can link together in multiorientations in order to achieve different patterns and configurations critically. This photo irradiation switching mechanism shows great potential for the applications in bionanotechnology such as remote and controllable drug release.

Based on the above studies, we strongly believed that photo-triggered release of drug molecules from multidimensional DNA-based nanocarriers would become a promising release mechanism and be highly achievable by careful designs. In an advance study, Han and coworkers have successfully introduced azobenzene moieties into 3D DNA tetrahedron (Figure 11(c)) [103]. Strands with introduced azobenzene groups can hybridize with the single-stranded hairpins, allowing the control of open and closed state of DNA tetrahedron by visible and UV light. The hybridization and dissociation of azobenzene-modified oligonucleotides can be remotely and reversibly controlled by the interconversion of *trans* and *cis* confirmations of azobenzene molecules.

FIGURE 11: (a) A design of photo-sensitive DNA nanodevice that make use of the properties of azobenzene towards different wavelengths of light. (b) Making use of the *cis-trans* properties of azobenzene under different wavelengths to close or disclose the active site of enzyme. (c) The shape of the azobenzene modified DNA tetrahedron can be altered in the presence of different wavelengths.

It is believed that these studies will open doors to implement and facilitate the 3D structural changes for triggered-release of encapsulated cargos in DNA-based nanoobjects.

6. Cellular Internalization and Site-Specific Targeting of DNA Nanostructures

6.1. Passive Delivery. DNA-based molecules usually have great difficulties in delivering to cells as they are highly negatively charged. They are not able to pass through cell membranes directly. Most of them undergo three types of possible mechanisms of getting in cells, Clathrin-mediated endocytosis, Caveolae-mediated endocytosis, and macropinocytosis. In general, Clathrin-mediated endocytosis is a type of endocytosis which requires excitation of receptor. The molecules would then be trapped in early endosome, then in late endosome, and finally in lysosome. The pH in a cellular environment is gradually decreased and then degradation of self-assembled DNA nanostructures is highly possible. Caveolae-mediated endocytosis is another type of endocytosis but it would go to Caveosome and then migrate to Golgi, endoplasmic reticulum, and endosomes. Macropinocytosis is different from the above two endocytic pathways as it is nonspecific. Though the molecules should end up at lysosome but the macropinosome is comparatively leaky which make

them possible to enter the cytosol to escape the destiny of degradation [104–106]. Efforts have been put to improve the cellular uptake of DNA-based nanomaterials in terms of high cell penetration ability and low cytotoxicity [107, 108].

6.2. Targeting of Self-Assembled DNA Nanostructures. To enhance the selective delivery of DNA nanocarriers to cancer cells or particular intracellular organelles for drug delivery purposes, a targeting moiety has to be conjugated to DNA assemblies.

6.2.1. Folate. Folate, water-soluble vitamin B₉, has proven to be an efficient targeting agent for cancer cells as folate receptors are overexpressed on the surfaces of cancer cells. Therefore, DNA nanostructures decorated with folate group via a simple NHS chemistry would provide a higher chance to be taken up by cancer cells over normal cells. Mao's group integrates folate into his DNA nanotubes (Figure 12(a)) [51]. They prove that the folate modified DNA nanotubes enter KB cells through overexpressed folate receptor and be able to internalize in the cells. One hour incubation of these modified nanotubes would be saturated because cells may only be able to take up certain amount of DNA nanotubes. When the folate content in the DNA nanostructures reaches 10%, the uptake capability of DNA nanotubes in cells would reach plateau due to the limited number of folate receptors.

FIGURE 12: (a) Cy3 and folate is covalently conjugated to the ssDNA via NHS chemistry for cell targeting and visualization. (b) The figure is showing the design of the DNA icosahedral nanoparticles and the possible releasing mechanism of doxorubicin.

6.2.2. Aptamer. In general, aptamers are short, single-stranded nucleic acid strands with specific sequences derived from systematic evolution of ligands by exponential enrichment (SELEX). They are able to recognize and bind to cellular surface receptors in certain cancer cells and thus allow importing to the cells, leading to target delivery. Huang's group have designed a DNA icosahedra from a six-point-star motif with a sticky end segment of MUC 1 aptamer sequence (Figure 12(b)) [109]. MUC 1 is a major class of tumor surface marker which is abundant on the surface of most epithelial cancer cells [110, 111], serving as entering portals for aptamers [112]. To investigate the targeting selectivity, the

uptakes of DNA polyhedron by MCF-7 cells which are MUC-receptor positive tumor cells, and by CHO-K1 cells which are MUC-receptor negative cells, have been investigated. They found that aptamer-modified DNA polyhedra exhibited higher cellular internalization efficiency than the regular DNA polyhedra do in MCF-7 cells but not in CHO-K1 cells, confirming an aptamer-mediated cellular selectivity of internalization of DNA polyhedra. They have proposed a cellular uptake mechanism for aptamer-modified DNA polyhedra in MCF-7 cells. First, MUC-modified DNA polyhedra recognize MUC 1 which is then rapidly recycled through intracellular compartments. After that, MUC-modified DNA polyhedral

FIGURE 13: (a) DNA strand are modified to bind with different functional domains and photosynthesized to a bigger complex. The nanostructure contains aptamer for differential cell targeting. (b) A design of label-free fluorescent probe incorporated in DNA origami.

structures are smuggled to endosome and later to lysosome by binding to MUC 1.

Tan's group have successfully designed and generated multifunctional DNA nanoassembly by first self-assembling three components, including aptamer, acrydite-modified ssDNA, and antisense oligonucleotides to form Y-shaped DNA domains (Figure 13(a)) [70]. Subsequently, these functional DNA domains were hybridized to an X-shaped DNA connector to form building units. After photo irradiation, all building units were cross-linked to form aptamer-based DNA assemblies. In this study, sgc8 aptamer and KK1B10 aptamer were chosen to demonstrate the generality of selective recognition of target cancer cells by these multifunctional aptamer-based nanoassemblies. Their results indicated that sgc8-functionalized DNA assemblies internalized specifically to CCRF-CEM cancer cells (T cell acute lymphoblastic leukemia cell line) but not to Ramos cells (B cells human Burkitt's lymphoma). While KK1B10 can specifically recognize and internalize into K562/D (Dox-resistant leukemia cell line)

but cannot control Ramos cells. Using this technique, the construction of the nanocarrier is easy to achieve and is highly programmable as the position, number, and size of the aptamer can be adjusted. In addition, this system has been tested *in vitro*, indicating that the nanoassembly is enzymatic resistant and cytotoxic negligible.

Recently, Kim et al. decorated their L-DNA nanocarriers with antiproliferative aptamer, ASI411, allowing them to selectively recognize and take up by cancer cells [86]. This is likely due to the interaction between ASI411 aptamers on L-DNA nanocarriers and the target protein nucleolin expressed on the surface of HeLa cells.

6.2.3. Organelle Localization Signal Peptides. Most of the self-assembled DNA nanostructures are taken up and eventually localized in lysosomes, endosomes, or Golgi networks by means of endocytosis (Figure 13(b)) [75, 95, 113]. It is realized that these locations are highly limited by their biological

FIGURE 14: (a) Demonstration of how functionalized vertical silicon nanowire arrays help in direct delivery of molecules to cytosol. (b) The triangular prism has been attached to MTS and NTS for specific cell internal targeting to mitochondria and nucleus, respectively. (c) Cy5-labeled MTS DNA-NCs with MitoTracker green and Cy5-labeled. (Scale bar represents 15 μ m).

behaviors and functions in a cellular system among different intracellular compartments.

Our group recently developed a new delivery technology on the basis of functionalized vertical silicon nanowire arrays as a delivery platform to transport intact DNA cages to the cytosol efficiently without endocytosis (Figure 14) [52]. We proved that this delivery strategy exhibits high cellular uptake efficiency together with great stability and low cytotoxicity in a cellular environment. In addition, this delivery approach would preserve the structural integrity of cages and help them escape degradation under endocytosis. More importantly, we demonstrated the first example of site-selective DNA nanocages for targeting mitochondria and nuclei. In this study, specific organelle localization signal peptides such as mitochondrial localization signal (MLS) peptide or nucleus

localization signal (NLS) peptide were incorporated to one of the constituent DNA strands and then further assembled to MLS or NLS peptide-functionalized DNA nanocage. It is found that the modified MLS or NLS-cages are able to localize exclusively in mitochondria or nuclei, respectively, by means of a powerful SiNW delivery platform *in vitro*. This work opens a door for the use of DNA nanocage as smart vehicles, particularly for targeted drug delivery to the specific intracellular organelles.

7. Conclusions and Outlook

DNA nanotechnology becomes a cutting edge research in recent years. The role of DNA in nanotechnology has reached far beyond its intrinsic role in biology. With the well-known

knowledge of self-recognition properties of DNA and its double helix feature on the molecular level, different geometries and sizes of DNA-based nanoarchitectures can be generated very accurately and efficiently in contrast to other self-assembling systems. In this review article, we summarized recent progress of drug delivery system based on multidimensional DNA nanostructures. Thus, self-assembled DNA nanostructures are undoubtedly highly promising scaffold to act as a drug nanocarrier or to display functionalities for therapeutic applications. From the high demand of multifunctional DNA carriers in the context of drug delivery vehicles that have been described in detail here, we can summarize several reasons why self-assembled nucleic acid structures are feasible for targeted drug delivery. First, the DNA nanostructures can be designed and modified with multifunctional groups including drug molecules, targeting motifs, and fluorescence probes and position all of them with high accuracy. Second, in comparison with multistep synthesis of other nanocarrier scaffolds like dendrimers, the desired DNA nanoobjects with great versatility can be easily formed by simple mixing of individual DNA building blocks together in a single step. This strategy can be achieved a large size of DNA nanostructures effortlessly, ranging from only a few nanometers to micrometer scale. Another conspicuous feature suggesting the use of self-assembled DNA-based carrier is that they can pass through the negatively charged plasma membrane and get into the cells efficiently without the need of transfecting agents, except some of the large and flexible DNA origami structures as compared to naked DNA strand itself. In addition, all DNA-based nanomaterials exhibited a very low cytotoxicity, no matter in the presence or absence of the payloads or stimuli. Such feature makes the self-assembled DNA nanoarchitectures a promising delivery system. Another striking advantage of using DNA nanoobjects for the purpose of drug delivery is that a large number of drug loading methods have been utilized for the interaction between drug molecules/cargos and self-assembled DNA nanostructures. We have described the examples briefly in this paper. They included covalent linkage, nucleic acid base-pairing, biotin-streptavidin interaction, intercalation, aptamer-targeted interaction, DNA-protein interaction, and encapsulation. Scientists also demonstrated several possibilities for the control release of drug or cargo molecules. In the presence of the specific and weak hydrogen bonds between A and T, and C and G nucleobases, a strand displacement is a method by adding an eraser DNA or RNA strand, allowing exchange and release of strands consisting of a toehold overhang. This DNA-mediated release strategy highly relies on specific nucleic acid sequences. When those DNA nanostructures are introduced into an environment with different pH values, i-motif switching is a promising mechanism for structural change and control release of cargo simultaneously. Another option for drug release is the use of light. In this case, light acts as a stimulus to facilitate the clean removal process. No accumulation of waste happens. Overall, multifunctional DNA nanostructures have successfully demonstrated their efficient intracellular delivery and specific targeting to cancer cells or particular intracellular organelles including, lysosomes, endosomes, Golgi networks,

mitochondria, and the nuclei. They are also extensively used for the delivery of certain drug or cargo molecules in living cell systems and induced some cellular activities or effects accordingly. To sum up, self-assembled DNA nanostructures offer unprecedented control over their structures and functionalities in a biological or cellular environment, the above examples demonstrate the potential applications, particularly for targeted drug delivery or gene regulation.

However, the use of DNA nanostructures in the biomedical field faces several challenges. As self-assembled DNA nanostructures have been seriously considered for the application in drug delivery, further studies are needed to obtain better information for their practical applications. These consist of the understanding of cellular uptake mechanism such as their intracellular pathway and pharmacokinetics. Can they escape from the fate of being degraded by endocytosis before reaching the target sites and taking biological effects? It is also necessary to investigate the relationship between their intracellular behavior/function and their various chemical/physical properties such as functional group incorporation, surface charges, nucleobase sequences, geometry, and dimensions. Another focus which should be concentrated on is the study of selective targeting of functionalized DNA nanostructures in terms of discrimination of diseased cells from common normal cells *in vitro* and *in vivo*. For instance, how can they be only taken up by cancer cells, but not macrophages? It is also important to look for some chemical modifications to prevent the formation of aggregates in circulating system and overcome the multilayers barriers after the DNA-based nanocarriers enter human body. The last but not the least, an alternative new and safe control release mechanism for drug molecules should be developed such that no waste is accumulated in biological system in addition to no harm being induced to the tissues of human bodies. We strongly believe that these suggested questions and studies are attractive topics to be investigated in the near future.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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