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Introductions of Nucleic Acid-Based Nanomaterials

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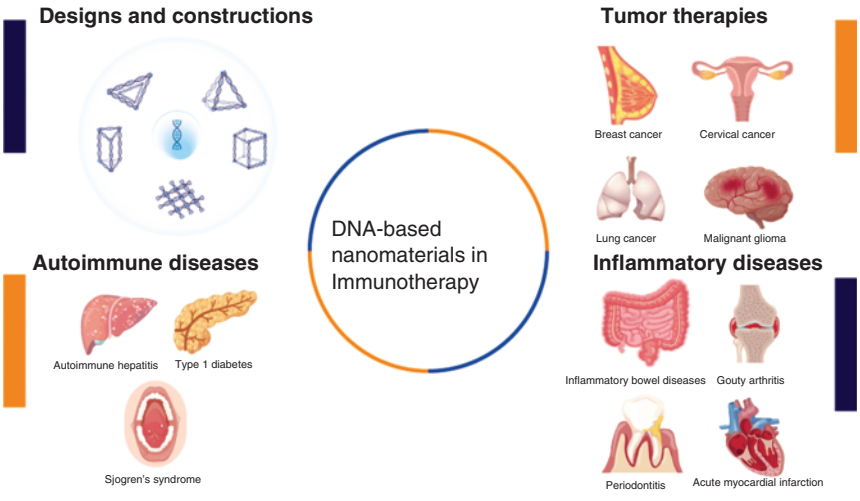
Nanotechnology is a science and technology that produces substances from a single atom or molecule in a size range of 1–100 nm. As early as 1986, American scientists put forward nanotechnology in the creation of machines, but due to the low level of science and technology at that time, the technology did not achieve obvious results. Researchers believe that nanotechnology is to make the combination of molecules in a machine practical, so as to arbitrarily combine all kinds of molecules to produce different molecular structures. Nanotechnology has been extensively applied in various fields and has had a profound impact on our lives. The concept of nanotechnology was first introduced to the public in the speech "There's Plenty of Room at the Bottom" in 1960 by Nobel Prize laureate Richard P. Feynman. With the continuous development of science and technology, people's research on nanotechnology is also in-depth, and the corresponding branch of the subject has also developed. Nanotechnology integrates quantum mechanics, molecular biology, nanobiology, nanochemistry, and other disciplines with the ultimate goal of directly constructing products with specific functions from atoms or molecules. Nanotechnology transformed the drug delivery system dramatically by delivering microtherapeutic drugs to parts of the body that are difficult to reach otherwise. With an expanding array of strategies that allow nanomaterials to tailor their properties to specific indications, nanomaterials are entering the clinic at an unprecedented rate [1]. In nanotechnology, nanomaterials are often defined as the creation of materials with new properties and functions at the nanoscale. There are two main approaches of constructing nanomaterials so far: the "top-down" approach is to reduce the size of large structures to nanoscale, while the "bottom-up" approach, which is also called "molecular nanotechnology," is to engineer materials from molecular or atom components through assembly or self-assembly. In 1953, the discovery of deoxyribonucleic acid (DNA)'s complementary base pairing principle and double helix structure ushered in the era of molecular biology. DNA became a potential material for nanofabrication due to its unique properties and high controllability [2–4]. Seeman and his coworkers first reported the synthesis rules of DNA-based nanomaterials in the 1980s, bringing DNA nanotechnology into the limelight as a research hotspot. The most common approach to building DNA-based nanomaterials is the "bottom-up"

Nucleic Acid-Based Nanomaterials: Stabilities and Applications, First Edition.
Edited by Yunfeng Lin and Shaojingya Gao.

approach. In recent years, DNA-based nanomaterials have been widely used in bioimaging, biosensing, gene transfer, drug delivery, disease diagnosis, and treatment due to their inherent biodegradability and biocompatibility. In addition, DNA-based nanomaterials are easy to customize in size and shape and have good structural stability.

DNA-based nanomaterials come in a variety of sizes and shapes and are designed in a variety of structures, including two-dimensional and three-dimensional structures. According to different molecular construction methods of functional DNA-based materials, synthetic DNA-based nanomaterials include monolayer and multilayer nanomaterials. These DNA-based nanomaterials can also be divided into circular, linear, and branching forms, and they have been extensively constructed and studied [5]. DNA-based nanomaterials were commonly treated as drug delivery systems. In immunotherapy, traditional materials, including liposomes and adenoviruses, have been used as drug delivery systems in the past, but their defects limit their clinical application. For example, they share the same disadvantage of low targeting ability. Separately, adenoviruses are difficult to build and are usually toxic, while liposomes are easy to build but have low portability and low toxicity. Compared with traditional materials, DNA-based nanomaterials have many advantages, including structural stability, unparalleled programmability, natural biocompatibility, and negligible immunogenicity. These advantages may make DNA-based nanomaterials more favorable in immunotherapy.

DNA-based nanomaterials with different structures are made for different biomedical purposes. With the development of DNA nanomaterials advancing, various new DNA-based nanomaterials were constructed and widely used in immune engineering, drug delivery, molecular biology, tissue engineering, disease diagnosis or biosensing, etc. [6–18]. In recent years, successful attempts in immunotherapy have been reported, suggesting that DNA-based nanomaterials may possess therapeutic potential. Other hyper-polymeric compounds and nanomaterials such as avidin [19], polyethylenimine (PEI) [20–22], chitosan [23–25], and gold nanoparticles (AuNPs) [26–28] were loaded on DNA-based nanomaterials to enhance their therapeutic effect. It has been reported in previous research that, together with other materials or specific structures, polymeric DNA-based nanomaterials could influence the biological behavior of cells, such as proliferation [8, 9, 11, 29], autophagy [10], differentiation [30, 31], cell viability [29, 32], morphology [33], and migration [34]. Thanks to these special properties, polymeric DNA-based nanomaterials can be potential treatments for certain diseases and applied for tissue regeneration engineering [35–37]. When combined with other materials, such as proteins and some chemical drugs, polymeric DNA-based nanomaterials could treat some autoimmune diseases [38–41]. When combined with aptamers, the DNA-based nanomaterials could promote the antitumor effects [29, 42, 43], the inhibition of malignant cells [42], and the ability to target [29, 32, 42]. In the past few decades, DNA-based nanomaterials have made great progress and development, providing new options for the effective treatment of a variety of diseases and making significant contributions to the public health of society. At present, scientists have developed a variety of delivery systems, such as DNA origami, DNA tiles, and tetrahedral DNA-based nanomaterials (TDNs). In this chapter, we will make a summary of the self-assembly and structural design of DNA-based nanomaterials and highlight their therapeutic potential in immunotherapy.



1.1 History of DNA-Based Nanomaterials – Design and Construction

Nucleic acid is a biological macromolecule used by living organisms to store genetic information [4, 44]. Being the basic genetic material in nature, it is not only closely related to normal life activities such as growth and reproduction, genetic variation, and cell differentiation but also closely related to abnormal life activities such as the occurrence of tumor, radiation damage, genetic disease, metabolic disease, viral infection, and so on. Moreover, nucleic acids have many unique properties besides their biological function; their molecular recognition ability, biocompatibility, and controllability at the nanoscale contribute to the construction of a variety of complex inorganic and organic nanostructures. Therefore, the study of nucleic acids is an important field in the development of modern biochemistry, molecular biology, and medicine. Nucleic acids are usually found in cells in the form of nucleoproteins that bind to proteins. Natural nucleic acids are divided into two main groups, namely ribonucleic acid (RNA) and DNA. The high controllability and high precision of Watson–Crick base pairing made DNA-based nanomaterials a potential substance for nanofabrication [2–4, 45]. Through the process of self-assembly, a large number of DNA-based nanomaterials of different shapes and sizes have been designed and constructed based on the classical Watson–Crick base pairing principle. Some DNA-based nanomaterials can change the biological behavior of cells functionally, such as cell migration, cell proliferation, cell differentiation, autophagy, and anti-inflammatory effects; hence, DNA nanotechnology has been greatly developed. DNA-based nanomaterials are employed in different scientific directions for various biological applications such as tissue regeneration, disease prevention, inflammation inhibition, bioimaging, biosensing, diagnosis, antitumor drug delivery, and therapeutics. In this section, we hope to introduce you to a comprehensive history of DNA-based nanomaterials.

The era of molecular biology began in 1953 with the discovery of the principle of complementary base pairing and the double helix structure of DNA. Ever since then research on the genetic function of DNA at the microlevel has become a hot topic. In the year of 1990s, further research sparked new interest in nongenetic functions among nucleic acid researchers, prompting scientists to further investigate. The first discovered were DNA aptamers of thrombin and RNA aptamers of organic dyes, further discoveries increased the number of nongenetic nucleic acids; thus, the concept of "functional nucleic acids (FNAs)" was proposed.

FNAs is a general term for nucleic acids and nucleic acid-like molecular particles, such as aptamers, DNA tiles, DNAzymes, DNA origami, and other forms of unconventional nucleic acids that can function like traditional antibodies and proteases and perform specific biological nongenetic functions with independent structural functions [46–50]. FNAs are composed of several kinds of nucleotides and are easy to synthesize. There are more than a dozen base nucleotides, including A, G, C, T, X, and Y [51]. As more and more effort is being put into artificial nucleic acid synthesis, the technology of their synthesis becomes more sophisticated, and the cost has decreased. The structure of FNAs is diverse and can be expressed as single-stranded, double-stranded, three-stranded, and four-stranded DNA. Due to the high compatibility of FNAs, a variety of targets can be attached to them. Recently, FNAs have been widely used in molecular imaging, nucleic acid self-assembly, biomolecular detection, and other biological fields, showing great advantages [52]. In the following section, four different kinds of well-known FNAs will be introduced.

1.1.1 DNAzymes

DNAzymes are a class of DNA molecules with catalytic functions. Like protein and RNA-catalyzing enzymes, DNAzymes are capable of catalyzing many types of biochemical reactions and are extensively applied in asymmetric catalysis, biosensors, DNA nanotechnology, and clinical diagnostics [53]. Proteins are thought to be the only biological molecules that can function as catalysts; however, in the 1980s and 1990s, scientists discovered some RNA with catalytic properties, which prompted widespread research for DNA enzymes. DNAzymes are generally obtained through the SELEX (Systematic Evolution of Ligands by EXponential enrichment) *in vitro* screening technique. (The basic idea of SELEX technology is to chemically synthesize a large, randomized DNA library *in vitro* and mix it with the target substance. The complex of the target substance and nucleic acid is mixed in a solution, and after the nucleic acids that are not bound to the target substance are washed away, the bound molecules are separated. The nucleic acid molecules are then used as the template for PCR amplification and the next round of the screening process. By repeating the screening and amplification process, some DNA or RNA molecules that do not bind to or have low or medium affinity with the target substance are washed away. Adaptor proteins, namely DNA with high affinity with the target substance, are isolated from very large random libraries, and their purity increases with the SELEX process.) According to their different catalytic functions, DNAzymes can be divided into different categories. The discovery of DNAzymes is mostly exciting because of the realization of enzyme-free catalytic reactions, which overcame the dependence on natural enzymes. Therefore, DNAzymes have been widely used as switches in the fields of biosensors and bioimaging.

1.1.2 Aptamers

Aptamers are a series of single-chain nucleic acid molecules that bind to specific target molecules. Their specificity is similar to that of antibodies, and they have strict recognition ability and a high affinity for binding ligands. They first appeared in 1990, when two research groups reported using aptamers to target small ligands and proteins with high affinity [54]. Aptamers are constructed similarly to DNAzymes by using the SELEX method of random RNA or DNA sequence libraries. In the past decades, some progress has been made in the optimization of aptamers. The *in vitro* screening process was automated in the year of 2001, and then in the year of 2005, fluorescence magnetic bead-SELEX (FluMag-SELEX) technology was developed and applied for DNA quantitative analysis and aptamer selection. In 2010, cell-SELEX was introduced to generate aptamers that bind to specific cell types, which further improved the synthesis method of aptamers. Compared with antibodies, aptamers have significant advantages such as (i) target cells can be screened at low toxicity and low immunogenicity; (ii) better specificity and affinity than antibodies; (iii) easy to be chemically modified; (iv) can be easily and economically obtained; (v) good thermal stability; (vi) can be used in combination with other drugs for combined treatment; and (vii) have easily customizable properties, such as deletion, splitting, fusion, extension, and substitution, to improve performance, particularly as a result of the development of splitting aptamer technology, which enables the substitution of protein-based antibodies and the direct detection of small molecules. Due to their inherent thermal stability, aptamers can undergo multiple denaturations and regenerations. This makes them easy to regenerate and reuse when they are applied to a variety of biosensors. In the absence of a target, aptamers are indistinguishable from ordinary nucleic acids. The presence of the target induces a conformational change of the aptamer. When an aptamer is bound to some nanomaterials, conformational changes in the aptamer may separate some groups of nanomaterials and lead to charge changes and subsequent recorded potential changes. This feature has been used for electrochemical detection of various target types.

1.1.3 Triplex DNA

Triplex DNA was first introduced to the world in 1957 by Felsenfeld and Rich [55]. In the year of 1995, the structure of triplex DNA was described in detail [56], that is, based on the double helix structure, a third oligodeoxynucleotide can be combined in the large furrow region of double-stranded DNA by Hoogsteen hydrogen bond pairing to form triplex DNA. Each strand segment of the triplex DNA must be an all-purine sequence or an all-pyrimidine sequence, which is bound to the target double-stranded DNA to form triplex DNA oligodeoxynucleotides, called TFOs (triplex-forming oligonucleotides). At the beginning of the discovery of triplex DNA, due to insufficient evidence that triplex DNA could exist in the body, its actual biological significance did not draw much attention. It was not until Helene and coworkers [57, 58] confirmed that triplex DNA could be used for gene expression regulation that researchers began to show strong interest in triplex DNA. According to the composition and orientation of triplex DNA, intermolecular triplex DNA can be divided into three types: the first type, TC triplex DNA (C+·GC and T·AT triplets, parallel; A is adenine, G is guanine, T thymine, C is cytosine); the second type, GT triple-stranded DNA (G·GC and T·AT triplets, parallel or anti-parallel); and the third

type, GA-type triplex DNA (G·GC and A·AT triplets, antiparallel) [56]. In the last decade, triplex DNA has been widely used in sensing technology. These methods utilize triplex DNA not only as recognition elements but also as functional structure conversion units, allowing output signals to be generated during target recognition. Therefore, detection targets involving triplex DNA are not limited to specific nucleic acid sequences but cover a wide range of molecular targets, including antibodies, proteins, heavy metal ions, and small molecules.

1.1.4 DNA Origami and DNA Tiles

Among FNAs, DNA origami and DNA tiles are two components commonly used in nucleic acid self-assembly techniques to construct high-order nucleic acid nanomaterials. DNA tiles are completely dependent on the assembly of short DNA single strands, which are usually first assembled into unique or identical blocks and then further assembled into highly ordered finite structures. The sequence of each DNA block is related to the spatial position it occupies and is individually addressable [59]. In the 1990s, to construct interesting 2-dimension (2D) nanomaterials, scientists began designing DNA tiles with branches that resembled natural Holliday junctions [60, 61]. DNA-based structure nanotechnology was first proposed by Ned Seeman [62] and his coworkers in 1982. They creatively used specific sequences of DNA molecules to build out stable four-arm nanostructures (Holliday juncture/junction), which opened the structure of DNA nanotechnology to this new field of science. Since then, various nanostructures based on DNA self-assembly have been designed and constructed. They introduced the idea of branching DNA junctions and combining sticky end cohesion to manufacture geometric objects and periodic 2D lattices. By creating a structural motif with two four-way junctions, a set of branched complexes called double-crossed (DX) molecules is constructed. The proper adhesive end design enables DX molecules to perfectly self-assemble into periodic, two-dimensional lattices [63]. After further study, intersecting DNA tiles, triangles, and three-point star patterns were constructed into two-dimensional or even three-dimensional lattices, such as triple-crossovers (TX) and paramedic crossovers (PX) [64]. The development of DNA nanotechnology has benefited from the invention of DNA tiles. However, the synthesis of tile-based nanomaterials contains many interactions between short oligonucleotides. The synthesis output of nanomaterials requiring multiple reaction steps and purification is limited [51]. First reported by Rothemund in 2006, DNA origami, a DNA-based nanomaterial that uses a long strand of DNA as a microscaffold with many small fixed strands, has emerged to help solve the problems associated with the DNA tile method [51]. DNA origami is the folding together of scaffold strands (long DNA single strands) and hundreds of designed short DNA single strands. Each short DNA single strand has multiple binding domains with the scaffold strand, which are bound together by complementary base pairing and folded into arbitrary shapes in a manner similar to knitting. The DNA origami assembly scheme generally shows higher yield, stability, and ability to construct complex geometric shapes compared to the DNA tile-based assembly scheme. Researchers have used this technique to produce various 1D (1-dimension), 2D (2-dimension), and 3D (3-dimension) DNA nanostructures. The technology was first used to synthesize a number of monolayer and flat structures, such as triangles, simple rectangles, five-point stars, and some complicated graphics. All

the structures have their own unique sizes, about 100 nm in diameter, and the processes associated with the self-assembly as well as the design of such DNA-based nanomaterials were thoroughly described by Rothemund [51]. From then on, more related research has been inspired, and more two-dimensional graphics have been successfully synthesized, including a map of China. Currently, DNA origami self-assembled structures typically have a surface area of 8000–10 000 nm^2 and contain approximately 200 addressable points within this regional range, thus allowing researchers to design arbitrary structures and apply them to multiple fields.

After binding with various nanomaterials such as metal-based nanomaterials, carbon-based nanomaterials, silicon-based nanomaterials, bionanomaterials, magnetic nanomaterials, and other composite nanomaterials, FNA-nanomaterial composites are formed. Thanks to the exceeding advantages like targeting, signal conversion, and amplification capabilities of FNA, as well as the stability and versatility of nanomaterials, FNA-nanomaterial composites are mainly applied in several categories, which will be illustrated in the following section.

The biological imaging function of FNA nanomaterials has greatly promoted the development of early disease diagnosis and lesion imaging. Targeting and fluorescence recovery rate are the keys to FNA nanomaterial bioimaging. The basic principle of biological imaging is to focus on the specific affinity between the target (including tumor cells, proteins, metal ions, or specific DNA sequences) and the probe, as well as the effective delivery and recovery of fluorescent substances. Therefore, FNA nanomaterials fully meet the requirements of biological imaging. The nanomaterials prevent the degradation of FNA through the action of various nucleases in the body, providing the nanomaterials with the ability of targeting, fluorescence signal generation, and amplification. Some nanomaterials, like MNPs, also have photothermal effects that further activate the release of fluorescent substances.

Among the many applications of FNA nanomaterials, the most common one is biosensing. Biosensing can be divided into four steps: signal perception, signal conversion, signal amplification, and signal output, each of which could involve FNA nanomaterials. In the process of signal perception, the ability of targeting and decoding ensures the accurate acquisition of the target, while in the signal conversion step, various chemical groups could be labeled using a number of fluorescence signatures of FNAs. Various types of biosensors have been constructed due to the fluorescence and luminescence properties of various nanomaterials and FNA nanomaterials' ability to convert abstract molecular signals into realistic visual, fluorescent, or electrochemical signals. Also, the cleavage and extension of FNA strands are of great help in enabling biosensors to realize the signal amplification process. In the signal output step, according to the different detection principles, sensing process, and sensitivity, FNA nanomaterial biosensors could be divided into visual biosensors, fluorescent biosensors, and electrochemical biosensors.

FNA nanomaterials can be widely applied in the biomedical field thanks to their ability of drug delivery and molecular recognition. They are mainly applied in disease diagnosis and treatment [65]. Nanomaterials like AuNRs, AuNPs, and magnetic NPs are well-known for their advantages and optical properties in biocoupling, synthesis, focal imaging, and photothermal therapy that could prove of great benefit in biomedical applications. FNAs combined with such substances have been extensively explored for the diagnosis of diseases by scientists [66–68]. Self-assembled 3D FNA nanostructures, such as DNA origami and DNA

hydrogels, have good biocompatibility, stability, flexibility, and precise programmability, as well as switching characteristics and ease of synthesis and modification [6]. Thus, FNA nanomaterials are expected to have potential in areas such as disease analysis and drug delivery [69, 70].

Today's world has completely entered the era of big data, and all life-related activities involve data storage and processing [71, 72]. The exponential growth of modern data has outpaced the capacity growth of existing memory devices. However, existing storage media, such as magnetic storage (magnetic tape or hard disk drive), optical storage (such as Blu-ray), and solid-state storage (such as flash memory), have been unable to meet the growing demand for storage capacity and have become a problem that human beings have to face. Molecular data storage is a novel data storage method with high stability and high storage density showing great potential. It is expected to address the growing gap between the amount of information available today and the capacity to store it. As a typical molecular data storage method, DNA data storage can be used as an alternative and transformative storage medium to break through the physical limit of existing storage methods and meet the ever-increasing demand for data storage. In recent days, a series of proof-of-principle experiments have demonstrated the feasibility and value of DNA as a storage medium, showing great potential for changing the way we store data [73, 74]. In a review reported by Panda et al., they critically analyze the emergence of the concept of DNA as a storage medium and its historical perspective, feasibility, recent breakthroughs, and the challenges that need to be overcome in order to make it a marketable data storage medium. They conclude that storing astronomical amounts of data in nucleic acids is no longer the stuff of science fiction [74].

After discovering that each DNA unit could bind with four adjacent DNA units or more, varieties of 3D DNA-based nanomaterial frameworks were manufactured. Ned Seeman's pioneering use of DNA as a building block to assemble high-dimensional materials has led to various methods of making DNA nanostructures of different sizes and morphs [75, 76]. One of the research focuses on making arbitrary structures in high dimensions, such as DNA paint fabricated using 2D tiles or micron-sized 3D DNA-based nanomaterials. Some three-dimensional DNA nanostructures with complex curvature have been prepared and characterized using DNA origami folding techniques [77]. For example, the double-helix DNA is bent to follow the circular outline of the target object, and potential chain crossings are subsequently identified. Concentric circles of DNA are used to generate in-plane curvature, constrained to two dimensions by appropriately designed geometry and crossover networks, resulting in a series of DNA nanostructures with high curvature and ellipsoidal shells. Interestingly, in 2009, Anderson et al. created an addressable DNA box that could be opened in the presence of externally provided DNA "keys" derived from the principle of complementary base pairing [78]. Controlling access to the inner compartments of such DNA nanocapsules could yield some interesting applications. At the same time, the closing and opening mechanisms of the DNA box have inspired researchers to build complex and multifunctional iterations of 3D nanorobots and nanocargo. As an example, the nucleolin-targeting aptamer serves as the targeting domain and as a molecular trigger that the DNA nanobots mechanically open. As a result, the internal thrombin is exposed and activates clotting at the tumor site. In later times, different kinds of 3D DNA-based nanomaterials were constructed. These DNA nanostructures can not only facilitate the study of molecular interactions in chemical and biological systems by building spatially organized molecular networks that can be used as molecular devices with more complex information

processing capabilities than before but also lead to the construction of more complex structural components in DNA robots and localized DNA circuits [79]. Douglas et al. demonstrated the design and self-assembly process of six different shapes of DNA nanostructures, including boulders, balustrade bridges, square nuts, stacked crosses, and monster bottles, with precisely controlled sizes ranging from 10 to 100 nm; he also reported on the effectiveness of the design approach by using honeycomb alignment to combine an integral, square mesh, and slotted cross [80]. In another study, Ke et al. reported a novel approach to the design of multilayered DNA structures based on quadruple-helix bundles. In this design, despite the high density of the DNA helix, the square lattice can be folded into nanostructures of the design size by a one-step annealing process [81]. With further study, Ke et al. reported the successful folding of a multilayered DNA structure, helically arranged on a tightly packed hexagonal lattice. The study also showed that hybrid DNA structures can contain three different shapes in a single design, including a square lattice, a honeycomb lattice, and a hexagonal spiral structure.

Although complex DNA origami technology provides a research platform and a versatile building block, its complex manufacturing process, high cost, relatively low yield, and technical sensitivity may limit its application unless breakthroughs are made in the production, folding, and purification of scaffolds. First introduced in 2004, Turberfield and his coworkers reported a convenient, one-step synthesis method of fabricating 3D DNA-based nanomaterials [82], which achieved progress in simple design, simple structure, low cost, and high yield. Of all the 3D DNA-based nanomaterials constructed, the DNA tetrahedron is supposed to be the simplest 3D DNA-based nanomaterial and one of the most classical 3D frameworks [83–85]. This ideal nanomaterial can be synthesized by a simple procedure in which four single strands of DNA are mixed in equal mole quantities in a saline buffer solution and denatured at 95 °C, then annealed by cooling to 4 °C. As the most typical 3D DNA-based nanomaterials, TDNs are composed of four isometric single-stranded DNAs and have strong mechanical strength as well as unique advantages over other types of DNA-based nanomaterials [60, 83, 86–90]. DNA, as a biological macromolecule, cannot enter the cell directly through the plasma membrane for its polyanionic nature, but because of its structural stability, it can be endocytosed by caveolin-mediated cells and then transported to the lysosome via a microtubule-dependent manner, meaning it can be maintained longer in cells [91]. In addition, TDNs can be specifically directed to targets that are capable of lysosomal escape when connected to nuclear loci aptamers, which may be key to gene delivery [92]. Zagorovsky et al. found that DNA-based nanomaterials possess better serum stability with more condensed spatial structure and higher DNA density, meaning that TDNs, due to their spatial simplicity, may be more stable than other structural 3D DNA-based nanomaterials [90]. Blessed with these specialties and with further discoveries, TDNs have become one of the most advanced nanomaterials in various fields.

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