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Review

Designer DNA nanostructures for therapeutics

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SUMMARY

The field of structural DNA nanotechnology applies the programmability of Watson-Crick base pairing to the construction of custom nanostructures that are prescribed by the sequence information encoded in DNA molecules. Precisely defined geometries, highly programmable molecular interactions, and outstanding biocompatibility make DNA nanostructures a new category of nanocarriers for drug delivery. Over the past decade, the potential of using DNA nanocarrier-based formulation for cancer therapy has been extensively explored with the successful implementation of various therapeutic strategies, both *in vitro* and *in vivo*. Moreover, DNA nanocarriers can be encoded with complex instructions via sequence design, enabling therapeutic functions to be executed in a programmed, automatic manner. In this review, we summarize recent advances and discuss the challenges and opportunities in designer DNA nanostructure-enabled therapeutics.

INTRODUCTION

Nanotechnology is an interdisciplinary research field focusing on the manipulation and organization of matter at the nanometer scale with the aim of achieving novel functions. In particular, nanomedicine, a branch of nanotechnology, takes advantage of the unique properties of nanomaterials for diagnostics and therapeutics.[1,2] It has been proven that including nanocarriers in therapeutic formulations can improve effectiveness and minimize adverse effects of conventional drugs.[1–3] By 2016, over 50 nanomedicines had been approved by the U.S. Food and Drug Administration (FDA), with many more undergoing clinical trials.[4] A majority of FDA-approved nanomedicines are based on micellar, liposomal, polymeric, and inorganic nanoparticles.[4] They are more or less limited by the heterogeneity in size and shape, chemical or physical instability, and potential cytotoxicity. Research on molecular self-assembly has suggested the prospects of using a programmable biopolymeric material, DNA, to enrich the choices of nanocarriers. Although at an early stage of development, the use of DNA nanocarriers to facilitate drug delivery has demonstrated profound potential (Figure 1).

DNA is the most widely used molecular building block for nanofabrication via self-assembly. Besides its biocompatible nature, DNA possesses several uniquely advantageous characteristics, such as a well-defined geometry, predictable and programmable complementarity, abundant choices of sequences, thermostability, affordable synthesis chemistry, and readily accessible chemical and enzymatic tools for modification. The history of using DNA as a nanoscale building material can be traced back to 1982 when Ned Seeman proposed the concept that "migrationally immobile junctions" could be assembled from rationally designed sequences and further joined together to form two-dimensional (2D) or three-dimensional (3D) networks through complementary single-stranded overhangs called sticky ends (Figure 2A).[5] The early work in structural

The bigger picture

The development of nanomedicine is closely related to the evolution of nanocarriers that endow therapeutic agents with improved efficacy, safety, and targeting specificity. A grand challenge in nanomedicine is the ability to design suitable nanocarriers that can precisely transport therapeutic agents across biological barriers in the complex environment of living organisms. The programmable and biocompatible nature of DNA has enabled the rational design of tailored DNA nanocarriers for targeted drug delivery, which has greatly contributed to the remarkable progress in cancer therapy. Additionally, DNA nanotechnology-enabled therapeutic systems have demonstrated profound potential in the development of smart therapeutic robotic systems and the treatment of renal diseases. In this review, we discuss the roles of DNA nanostructures in different therapeutic strategies and highlight model systems that hold promise for clinical translation in the long term.

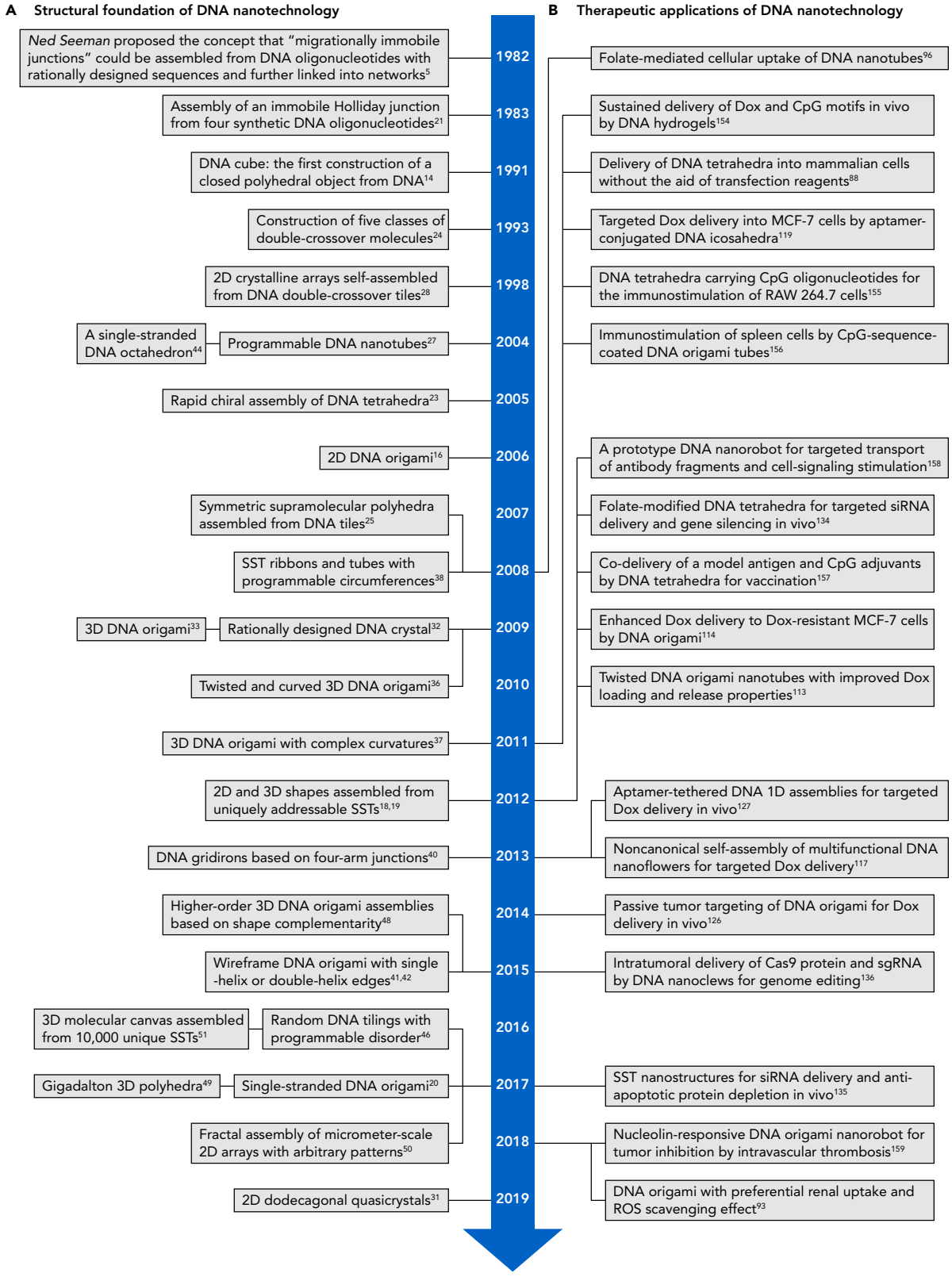
A Structural foundation of DNA nanotechnology
B Therapeutic applications of DNA nanotechnology
Ned Seeman proposed the concept that "migrationally immobile junctions" could be assembled from DNA oligonucleotides with rationally designed sequences and further linked into networks[5]
1982
Assembly of an immobile Holliday junction from four synthetic DNA oligonucleotides[21]
1983
DNA cube: the first construction of a closed polyhedral object from DNA[14]
1991
Construction of five classes of double-crossover molecules[24]
1993
2D crystalline arrays self-assembled from DNA double-crossover tiles[28]
1998
A single-stranded DNA octahedron[44]
Programmable DNA nanotubes[27]
2004
Rapid chiral assembly of DNA tetrahedra[23]
2005
2D DNA origami[16]
2006
Symmetric supramolecular polyhedra assembled from DNA tiles[25]
2007
SST ribbons and tubes with programmable circumferences[38]
2008
3D DNA origami[33]
Rationally designed DNA crystal[32]
2009
Twisted and curved 3D DNA origami[36]
2010
3D DNA origami with complex curvatures[37]
2011
2D and 3D shapes assembled from uniquely addressable SSTs[18,19]
2012
DNA gridirons based on four-arm junctions[40]
2013
Higher-order 3D DNA origami assemblies based on shape complementarity[48]
2014
Wireframe DNA origami with single -helix or double-helix edges[41,42]
2015
3D molecular canvas assembled from 10,000 unique SSTs[51]
Random DNA tilings with programmable disorder[46]
2016
Gigadalton 3D polyhedra[49]
Single-stranded DNA origami[20]
2017
Fractal assembly of micrometer-scale 2D arrays with arbitrary patterns[50]
2018
2D dodecagonal quasicrystals[31]
2019
Folate-mediated cellular uptake of DNA nanotubes[96]
Sustained delivery of Dox and CpG motifs in vivo by DNA hydrogels[154]
Delivery of DNA tetrahedra into mammalian cells without the aid of transfection reagents[88]
Targeted Dox delivery into MCF-7 cells by aptamer-conjugated DNA icosahedra[119]
DNA tetrahedra carrying CpG oligonucleotides for the immunostimulation of RAW 264.7 cells[155]
Immunostimulation of spleen cells by CpG-sequence-coated DNA origami tubes[156]
A prototype DNA nanorobot for targeted transport of antibody fragments and cell-signaling stimulation[158]
Folate-modified DNA tetrahedra for targeted siRNA delivery and gene silencing in vivo[134]
Co-delivery of a model antigen and CpG adjuvants by DNA tetrahedra for vaccination[157]
Enhanced Dox delivery to Dox-resistant MCF-7 cells by DNA origami[114]
Twisted DNA origami nanotubes with improved Dox loading and release properties[113]
Aptamer-tethered DNA 1D assemblies for targeted Dox delivery in vivo[127]
Noncanonical self-assembly of multifunctional DNA nanoflowers for targeted Dox delivery[117]
Passive tumor targeting of DNA origami for Dox delivery in vivo[126]
Intratumoral delivery of Cas9 protein and sgRNA by DNA nanoclews for genome editing[136]
SST nanostructures for siRNA delivery and anti-apoptotic protein depletion in vivo[135]
Nucleolin-responsive DNA origami nanorobot for tumor inhibition by intravascular thrombosis[159]
DNA origami with preferential renal uptake and ROS scavenging effect[93]

Figure 1. A timeline of the field and the emerging therapeutic applications of DNA nanotechnology

(A) A timeline of the field of structural DNA nanotechnology since 1982.

(B) A timeline of the emerging therapeutic applications of DNA nanotechnology since 2008. Representative discoveries and achievements are listed. SST, single-stranded tile; Dox, doxorubicin; ROS, reactive oxygen species.

DNA nanotechnology focused on exploring design principles and assembly methods for building complex structures (Figure 1A).[6–9] After more than 30 years of development, the structural complexity of DNA architectures has reached an unprecedented level. Research on the fundamental aspects of DNA-based self-assembly has facilitated the rational design of custom DNA nanostructures that can be tailored for specific purposes. In the past decade, numerous attempts have been made by DNA nanoscientists to use the DNA-nanotechnology-based platform for therapeutic applications (Figure 1B).[10–13] DNA nanostructures are primarily used as carriers to deliver therapeutic agents, including anticancer drugs, antisense oligonucleotides (ASOs), photosensitizers, nanoparticles, and proteins, to pathogenic sites. Moreover, other functional modules can be feasibly integrated into DNA-based nanocarriers, allowing for real-time tracking, precise targeting, conditional activation, and controlled release. The versatility of DNA nanostructures enables the implementation of different therapeutic strategies, including chemotherapy, gene therapy, phototherapy, and immunotherapy, both *in vitro* and *in vivo*. Additionally, the DNA-nanotechnology-based therapeutic platform provides new solutions to multidrug resistance (MDR), tumor vascularization, and acute kidney injury. The successful proof-of-concept applications in therapeutics have inspired ever-increasing efforts for "bench-to-bedside" translation.

In this review, we first introduce the fundamental design strategies and principles that have transformed the field of structural DNA nanotechnology. Criteria for developing DNA-based nanocarriers are then discussed in terms of stability, cellular uptake, targeting specificity, and economical manufacturing. In addition, recent advances in the therapeutic applications of DNA nanostructures are categorized and summarized based on their therapeutic strategies, including chemotherapy, gene therapy, phototherapy, immunotherapy, combination therapy, and therapeutic DNA nanorobots. As a case study, we discuss the potential use of DNA nanostructures for the treatment of kidney diseases. At last, we further discuss the challenges and potential future directions for the therapeutic applications of structural DNA nanotechnology.

STRUCTURAL DNA NANOTECHNOLOGY

Structural DNA nanotechnology exploits the hybridization of complementary DNA sequences to create structures via the self-assembly of DNA building blocks. A core aim of structural DNA nanotechnology is to use DNA nanostructures for the organization of other molecules and materials with nanometer precision in order to engineer novel functions. To this end, structural DNA nanotechnology initially focused on the exploration of structural motifs that could be rationally designed, which laid the foundation for the rapid evolution of the field since 2000s. In this section, we provide a brief overview regarding the fundamental design strategies and principles of structural DNA nanotechnology. Comprehensive reviews can be found elsewhere in the literature.[7–9]

Design strategies

Seeman's original concept of "migrationally immobile junctions" was validated soon after it was proposed through the assembly of an immobile Holliday junction from four synthetic DNA oligonucleotides with rationally designed sequences and prescribed complementarity.[21] Based on branched junctions, various covalently closed, discrete 3D objects, such as a cube[14] and tetrahedra,[22,23] were constructed

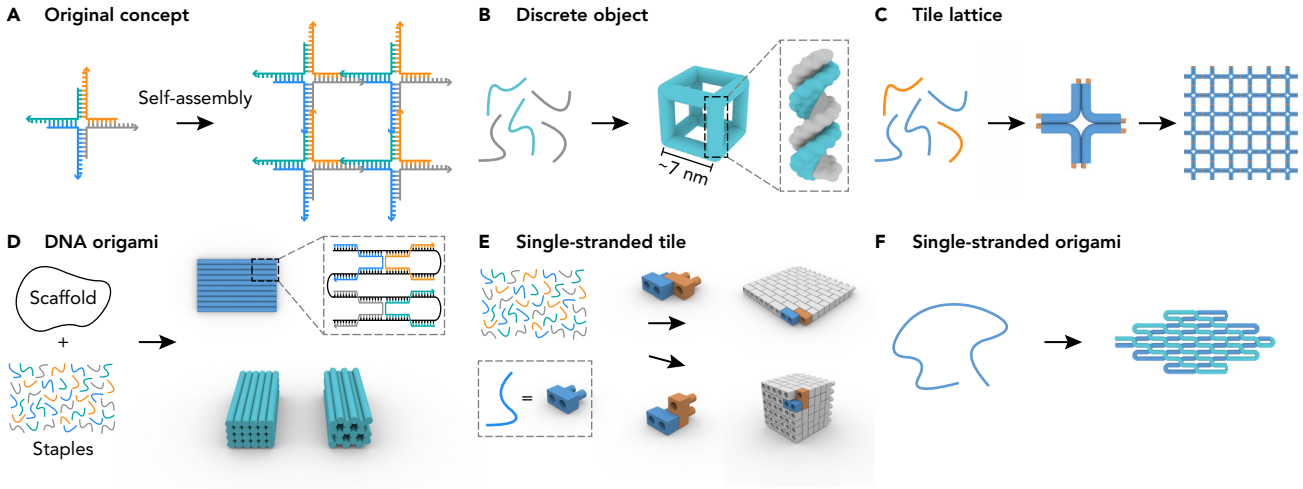

Figure 2. Structural DNA nanotechnology

(A) Ned's original concept: forming a 2D lattice from immobile junctions through complementary sticky ends.[5] Reproduced with permission from Seeman.[5] Copyright 1982 Elsevier.

(B) Discrete nanoobjects can be assembled from oligonucleotides with rationally designed sequences and complementarities. A ~7 nm DNA cube is the earliest polyhedral object constructed from DNA.[14]

(C) DNA tile-based self-assembly is a feasible approach to create higher-order structures from modular building blocks. For example, a 4-by-4 DNA tile can be assembled from several oligonucleotides and further linked into 2D lattices through complementary sticky ends.[15]

(D) DNA origami is the folding of a long, single-stranded DNA (termed "scaffold") into 2D or 3D shapes with the help of hundreds of short DNA oligonucleotides (termed "staples").[16,17] (Top right) Reproduced with permission from Rothemund.[16] Copyright 2006 Springer Nature. (Bottom right) Reproduced with permission from Castro et al.[17] Copyright 2011 Springer Nature.

(E) Single-stranded tiles (SSTs) can be programmed to assemble into 2D or 3D shapes without the guidance of a scaffold.[18,19] SSTs interact with each other through complementary domains, in a way analogous to "Lego" bricks. (Top right) Reproduced with permission from Wei et al.[18] Copyright 2012 Springer Nature. (Bottom right) Reproduced with permission from Ke et al.[19] Copyright 2012 AAAS.

(F) Single-stranded DNA origami is the unimolecular folding of a long single-stranded DNA into nanostructures through paranemic cohesions.[20] Reproduced with permission from Han et al.[20] Copyright 2017 AAAS.

(Figure 2B). Later on, modular DNA tiles[24] were proposed as nanoscale building blocks to construct extended, higher-order structures, including discrete nanocages,[25,26] nanotubes,[27] 2D arrays,[15,28–31] and macroscopic crystals,[32] via sticky end interactions (Figure 2C).

The groundbreaking technique of scaffolded DNA origami[16] was developed by folding a long single-stranded DNA (called a scaffold), typically derived from M13mp18 bacteriophage, into target shapes with the help of hundreds of auxiliary "staple strands" (Figure 2D). As an extension of this concept, 3D origami structures were designed by packing DNA helices into various 3D lattices.[33–35] Via targeted insertions and deletions of base pairs,[36] as well as curved-surface rendering,[37] DNA origami with complex curvatures can be reliably created. In the DNA origami design, each staple strand is uniquely addressable, which means it has a unique sequence and occupies a unique position that can be addressed as a "pixel" on the design, making DNA origami a versatile platform to organize other molecules with nanometer precision by placing them on these "pixels."

New strategies have further simplified the design of complex DNA nanostructures. Single-stranded tile (SST)[38] is the simplest form that a tile can take, as it is only composed of concatenated sticky ends. Complex structures, including 2D patterns,[18] discrete 3D objects,[19] and crystals,[39] can be constructed by sculpting or packing the molecular canvas formed by a large number of unique SSTs (Figure 2E). Methods for designing wireframe DNA origami with either single-helix[40,41] or

double-helix[42,43] edges offer new possibilities for developing DNA frameworks. The design of single-stranded DNA origami further enables a unimolecular folding of a single-stranded DNA of over several kilobases into discrete structures (Figure 2F).[20,44,45]

The size and complexity of DNA assemblies are further advanced by a series of scaling-up strategies. 2D random tilings,[46] 3D polyhedra,[47] dynamic devices,[48] and gigadalton-scale objects[49] can be built with high quality by assembling individual DNA origami into extended structures. Through a "fractal assembly" strategy, micron-scale, fully addressable arrays that displayed user-defined patterns were made from the hierarchical, multistage assembly of up to 64 unique components.[50] In the SST system, the molecular canvas assembled from SSTs was further expanded to up to 30,000 unique components.[51] Feasible methods for fabricating micron-sized DNA assemblies from a large number of unique building units will enable the development of molecular devices with sizes comparable with cellular organelles and provide the platform for various applications including biomimetics and therapeutics. In addition, DNA-based nanomaterials such as hydrogels and noncanonical nanoflowers have also been applied to biomedical applications and are discussed in this review.

Spherical nucleic acids (SNAs),[52] including DNA-functionalized inorganic nanoparticles, polymersomes, micelles, and liposomes, are distinct from the above-mentioned DNA nanostructures, as they are the covalent coating of a core material with a layer of highly oriented oligonucleotides. These groups of DNA-functionalized materials and their derivatives have been extensively researched and comprehensively reviewed elsewhere in the literature[52,53] and are not covered in this review.

Design principles

No matter how complicated the DNA structure is, the physiochemical principle it follows is the maximization of base-pairing events (i.e., reducing the thermodynamic free energy of the whole self-assembly system). Second, complex, higher-order structures can be produced from modular, branched building blocks. Moreover, given the noncovalent, reversible nature of DNA hybridization, choosing proper conditions could guide the self-assembly pathway to favor the rapid production of desired nanostructures with high yield.[54] Finally, dynamic properties such as reconfiguration, unidirectional motion, and reversible assembly can be integrated into structures via an enzyme-free strand displacement mechanism.[55–57] Overall, structural DNA nanotechnology offers tremendous design space for nanofabrication, setting the groundwork for diverse applications.

CRITERIA FOR DEVELOPING DNA-BASED NANOCARRIERS

An ideal drug-delivery system improves drug efficacy and safety by fine-tuning its retention and specificity *in vivo*. As a crucial component, the carrier for drug delivery should fulfill the following criteria: (1) it should be chemically inert and biologically stable in physiological environments; (2) be nontoxic, nonimmunogenic, and compatible with living organisms; (3) must have a controllable retention time and a clear clearance pathway; (4) should function specifically at the pathogenic site and have minimal effect on healthy tissues; and (5) be scalable and cost efficient. Synthetic DNA nanostructures do not inherently meet all the criteria, but they can be modified to better fulfill them. Moreover, the programmability of DNA-based platform is not found in any other systems, making it one of the most promising candidates for therapeutics. In this section, we discuss prior works on quantifying and

improving characteristics of DNA nanostructures with respect to the above-mentioned criteria.

Stability under physiological conditions

DNA nanostructures are usually prepared in buffers containing approximately 10 mM Mg^{2+} or 1.0 M Na^{+}, since cations can reduce charge repulsion between closely packed DNA helices, keeping structures from denaturation. Well-formed DNA origami can withstand repeated freezing and thawing cycles in buffer containing sufficient Mg^{2+} and cryoprotectants, facilitating long-term cryopreservation.[58] However, most DNA nanostructures are susceptible to low cation concentration and nuclease digestion in physiological environments.[59] The tolerance of DNA nanostructures to cation depletion and nuclease digestion is design dependent. In general, less compact structures can better withstand lower cation concentrations,[60] while more compact structures are more resistant to nuclease digestion.[17] For example, in contrast to a 24-helix bundle with a denser packing of DNA helices, a 6-helix bundle can be assembled in buffers containing less cations[60] and remain intact under low-Mg^{2+} conditions.[59,60,61] Wireframe origami can even be folded in Mg^{2+}-free buffers commonly used in biomedical research, such as phosphate-buffered saline (PBS).[41,43] Compared with duplex DNA or double-crossover tiles, paranemic crossover (PX) tiles with a higher number of crossovers showed enhanced nuclease resistance.[62] This effect can be attributed to local steric hindrance and global conformational strain and is in agreement with real-time visualization[63] and computational modeling[64] of DNA origami digestion by nucleases. Taken together, these examples suggest the possibility of tuning the stability of DNA nanostructures by rational design.

To maintain structural integrity under physiological conditions, it is necessary to protect DNA nanostructures against cation depletion and nuclease digestion with proper modifications (Figure 3A). Synthetic oligonucleotides with unnatural backbone, base, or left-handed chirality are suitable building units for assembling discrete or periodic nanostructures with improved serum stability.[65,66] However, their usage in stabilizing complex DNA nanostructures is usually hindered by the high synthesis cost. Chemical or enzymatic ligation of nick points[67,68] and photo-cross-linking of thymine bases[69] make individual components topologically interlocked for a better resistance against cation depletion and nuclease digestion. Decorating or coating DNA nanostructures with hexaethylene glycol (HEG),[70] lipid bilayer,[71] dendritic oligonucleotides,[72] peptoids,[73] peptides,[74] albumins,[75,76] and cationic block co-polymers based on oligolysine-polyethylene glycol (PEG),[77–79] poly(2-(dimethylamino)ethyl methacrylate),[80] chitosan, and linear polyethylenimine (LPEI)[81] helps shield them from nuclease digestion, greatly extending their half-life in cell-culture media (typically supplemented with 10% fetal bovine serum) or *in vivo*. Unlike DNA nanostructures, DNA-based nanoparticles (e.g., nanoflowers[82]) acquire nuclease resistance via a dense DNA packaging. Notably, single-stranded DNA origami has extraordinary thermostability,[20] as it is analogous to a fully ligated DNA structure. Its potential use as a drug carrier remains to be explored.

Cellular uptake

Despite the charge repulsion between DNA nanostructures and the cellular membrane, various DNA nanostructures can be readily internalized into mammalian cells via endocytosis. Cell type and the properties of DNA nanostructures such as size, shape, and surface chemistry are the major factors that determine the route of endocytosis. General trends derived from other materials can hardly be adapted to DNA-based systems due to the distinct surface chemistry. DNA nanostructures might be internalized into mammalian cells through multiple pathways, given that their sizes

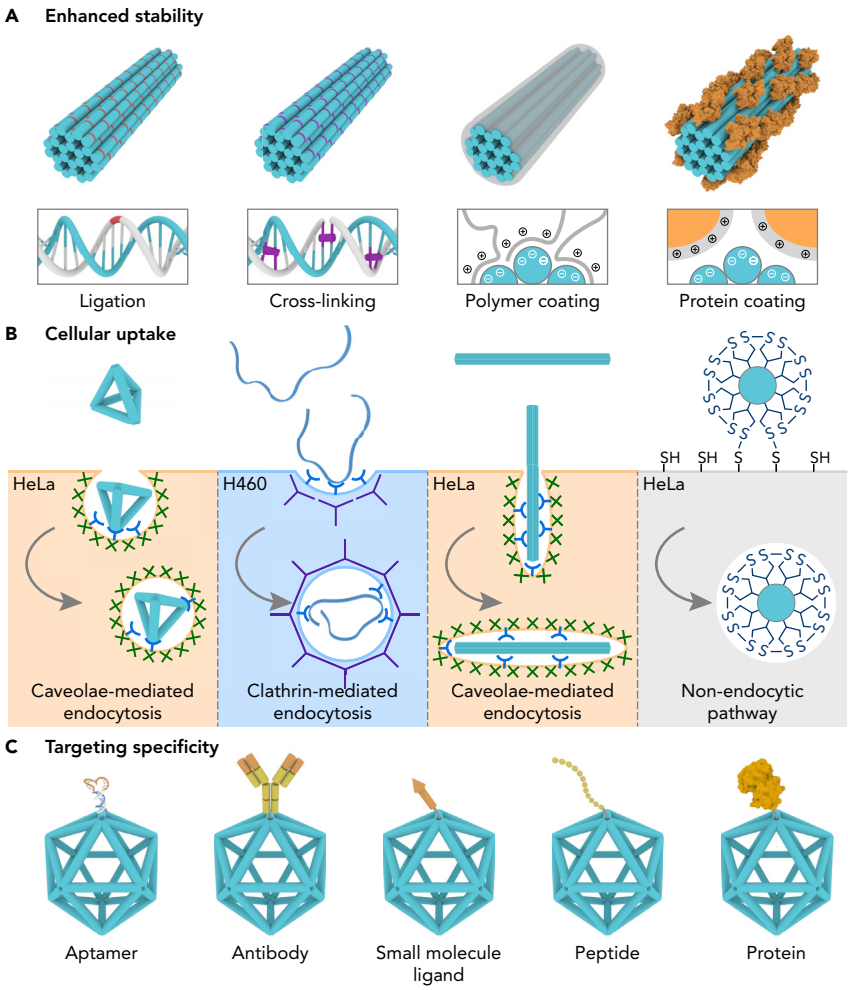

Figure 3. Criteria for developing DNA-based nanocarriers

(A) Common strategies to stabilize DNA nanostructures under physiological conditions. Unmodified DNA nanostructures are usually susceptible to cation depletion and enzyme digestion due to the charge repulsion between negatively charged DNA backbones and the existence of nick points between individual strands. Ligation of nick points, cross-linking, and surface coating are widely used strategies for protecting DNA nanostructures from denaturation or degradation under physiological conditions.

(B) Illustration depicting the cellular uptake pathways of DNA nanostructures. A DNA tetrahedron (7 nm) and a DNA origami rod (127 nm in length) are both internalized into HeLa cells via caveolae-mediated endocytosis.[83,84] A DNA nanoribbon with a high aspect ratio takes the clathrin-mediated endocytic pathway into H460 cells.[85] The disulfide-modified DNA nanospheres are directly internalized into the cytosol of HeLa cells through a nonendocytic pathway.[86]

(C) A variety of targeting modules, including aptamer, antibody, small-molecule ligand, peptide, and protein, have been integrated onto DNA-based carriers, resulting in improved targeting specificity and cellular uptake efficiency.

range from a few nanometers to the submicron scale (Figure 3B). A selection of recent studies focusing on the pathways of cellular uptake is discussed here. Comprehensive reviews on the cellular delivery of DNA nanostructures can be found elsewhere in the literature.[10,12,87]

A DNA tetrahedron was one of the earliest structures proven to enter mammalian cells without the aid of transfection reagents.[88] Internalized tetrahedra were found

localized in the cytoplasm and also substantially intact. As further elucidated by single-particle tracking,[83] caveolin-dependent endocytosis, followed by microtubule-dependent transportation to the lysosomes, was identified as the primary uptake and intracellular trafficking mechanism in HeLa cells. The underlying physical mechanism was obtained through a coarse-grained modeling of the tetrahedron-cell membrane interaction.[89] The modeling results suggested that tetrahedra predominantly attacked the spots of slight or no charge repulsion on the semifluidic membrane with their corners.

DNA origami can be folded into arbitrary shapes, facilitating a systematic evaluation of the effect of size and shape on endocytosis. Screening the shape-dependent uptake in three cell lines representing endothelial, epithelial, and immune cells uncovered a general trend wherein larger origami with more compact helical arrangement could be more efficiently internalized than smaller, less compact ones.[90] A similar trend was observed in lung cancer cell lines: larger origami were more preferred than smaller ones; compact, rod-shaped origami were more efficiently internalized than branched, tetrahedron-shaped ones.[84] The detailed cell-internalization process of the large rod was visualized in the latter study, which was mediated by scavenger receptors and was likely to follow a caveolin-dependent pathway to late endosomes and lysosomes.[84] This result is consistent with the co-localization of tubular origami and lysosomes in NIH 3T3 cells observed by super-resolution fluorescence microscopy.[91]

DNA assemblies can also be internalized via clathrin-mediated[85] or nonendocytic pathways.[86] However, not many different shapes have been tested for their internalization pathways. Moreover, cellular uptake is usually quantified by fluorescence-based techniques, which can be affected by a number of factors, including degradation of DNA structures, cellular uptake of fluorophores, and local environmental changes.[92] Given the fact that cell types differ vastly regarding their uptake efficiency, there are no comprehensive rules to predict the entry, biodistribution, and fate of DNA nanostructures. Further research is necessary to unravel the complex mechanisms of cellular uptake and to seek non-endo/lysosomal pathways to circumvent the acidic environment in the lysosome for the efficient delivery of therapeutic agents.

Targeting specificity

Studies on the *in vivo* distribution of DNA nanostructures in healthy mice suggest the preferential accumulation in liver or kidney after intravenous injection into the blood circulatory system.[71,93,94] Then, DNA nanostructures are degraded or excreted within 24 h. Encapsulating DNA nanostructures with PEGylated lipid bilayers can help retain over 80% of the administered dose throughout the mice as visualized 2 h after the initial injection.[71] Topical medication is another potential solution to circumvent rapid clearance in systemic administration. Transdermal drug delivery is especially suitable for skin cancer treatment and has been applied to the mouse melanoma model with initial success.[95]

DNA nanostructures interact with cells primarily through receptors displayed on the cellular membrane. Specific targeting and enhanced cellular uptake can be achieved by tagging DNA nanostructures with ligands that can be recognized by the receptors (Figure 3C). For example, folate modification enabled the specific internalization of DNA nanotubes into KB cells.[96] Enhanced cellular uptake of a rectangular DNA origami by HEK293 cells was facilitated by virus-capsid protein coating.[97] In addition, DNA aptamers that are selected via cell-SELEX[98] (systematic evolution of

ligands by exponential enrichment) are widely used as targeting modules, as they can be incorporated into most DNA nanocarriers. Furthermore, the cell entry and transport pathways of DNA nanocarriers can be readily modulated by varying the targeting molecules.[99]

The endocytic pathway usually determines the destination of intracellular transport. Most DNA nanostructures and their cargo are ultimately transported to lysosomes for degradation. Thus, endolysosomal escape is necessary so that DNA nanostructures can circumvent the acidic interior of lysosomes. DNA nanostructures can be modified with a secondary transport signal in order to be re-directed to target other organelles. For example, signaling peptides were anchored onto a DNA tetrahedron to facilitate nucleus targeting.[83] Modifying DNA nanostructures to take nonendocytic or endosomal escape pathways is a key strategy for targeting specific organelles which remains to be explored.

Economical manufacturing

High synthesis cost and long reaction time are two obstacles that hinder the rapid production of complex DNA nanostructures in large quantities. Biotechnological mass-production methods based on enzymatic amplification or bacteriophage replication have facilitated large-scale synthesis of replicable DNA nanostructures,[20,45,100,101] and single-stranded DNA that can be used as the scaffolds[102–104] or staples[102,105,106] for assembling DNA origami. Remarkably, by including self-excising DNAzyme sequences in the single-stranded precursor DNA produced by bacteriophages, target DNA can be produced without the need for costly restriction endonucleases.[107] The production cost of folded origami is estimated to be ~$25 per milligram for a liter-scale lab setup and even lower for a pilot-scale setup.[107] As a highly cooperative process, the folding of a DNA origami can be completed at a constant temperature within minutes.[54] Taking advantage of this characteristic, rapid and scalable self-assembly of DNA origami can be carried out with widely available lab equipments.[108] Moreover, scalable methods for scaffold purification by selective polymer catch and release[109] and for origami purification by rate-zonal centrifugation[110] and PEG-induced precipitation[111] further reduced the cost of preparing pure DNA nanostructures. Methods for economical and scalable synthesis, fabrication, and purification will benefit applications that require DNA nanostructures in large quantities and high purities, including therapeutics.

DNA-NANOTECHNOLOGY-ENABLED THERAPEUTICS

With a better understanding of their behaviors under physiological conditions and interactions with cells, DNA nanostructures have been extensively explored as a multifunctional platform to deliver therapeutic agents for the implementation of various *in vitro* and *in vivo* cancer-therapeutic strategies. In this section, we summarize these strategies and highlight those successful examples demonstrated *in vivo*.

Chemotherapy

As the most widely used strategy for cancer treatment, chemotherapy is conducted by delivering cytotoxic therapeutic agents to their functional sites. DNA-based drug-delivery systems use DNA nanostructures or DNA-based nanoparticles as carriers to improve the biodistribution, specificity, and efficacy of conventional drugs. The safety concerns on using DNA nanostructures can be precluded with appropriate design of DNA sequences (e.g., obviating the use of functional DNA modules like AS1411, a 26-base guanine-rich DNA aptamer with potential apoptotic induction activity). To date, many DNA-based carriers have been proven to have little cytotoxicity and are primarily used as the hub to integrate drug loading, targeting,

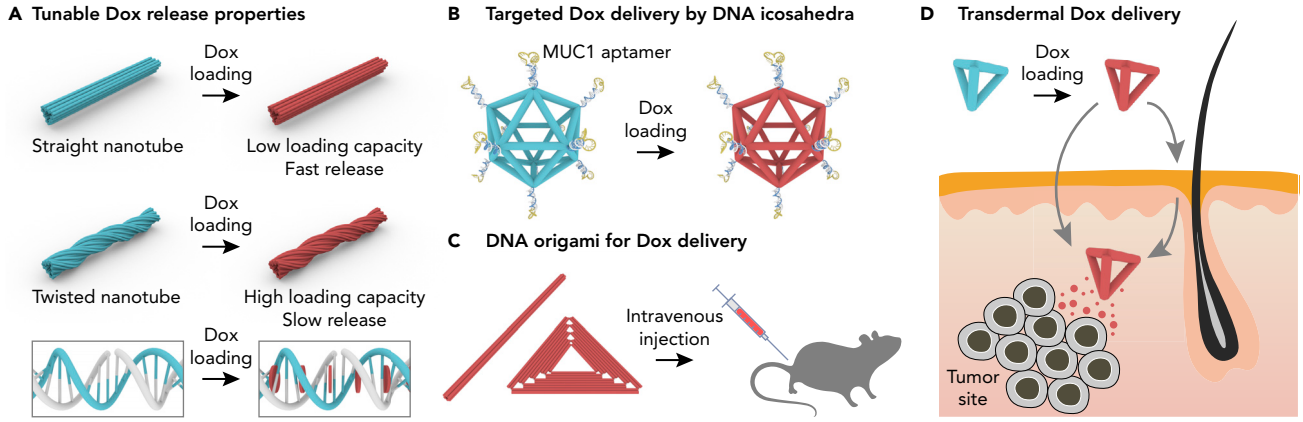

Figure 4. DNA nanotechnology for chemotherapy

(A) Tuning the loading capacity and releasing rate of Dox by varying the global twist of DNA origami nanotubes.[113] Dox can be easily loaded onto DNA nanostructures by intercalating between base pairs. Twisted DNA origami nanotubes with 12 bp/turn showed a higher loading capacity and a more sustained release, compared to straight ones with 10.5 bp/turn. Reproduced with permission from Zhao et al.[113] Copyright 2012 American Chemical Society.

(B) MUC1 aptamer-modified DNA icosahedra for targeted delivery of Dox into MCF-7 cells.[119] Reproduced with permission from Chang et al.[119] Copyright 2011 American Chemical Society.

(C) DNA origami for Dox delivery *in vivo*.[126] Dox-loaded DNA origami showed passive tumor accumulation and antitumor efficacy without observable systemic toxicity. Reproduced with permission from Zhang et al.[126] Copyright 2014 American Chemical Society.

(D) DNA nanostructures for the transdermal delivery of Dox.[95] The 17-nm DNA tetrahedra enabled the Dox to reach ~400 μm beneath the surface of mouse skin. Reproduced with permission from Wiraja et al.[95] Copyright 2019 Springer Nature.

and release modules together. Doxorubicin (Dox) is widely used as a model drug as it can be easily loaded onto double-stranded DNA by intercalation and released by disrupting the DNA duplex. Daunorubicin is another intercalating drug that has been delivered in a similar way. Other common model drugs, such as floxuridine (FU), paclitaxel (PTX) and camptothecin (CPT), are usually loaded onto the DNA carriers by conjugation or encapsulation.

Some DNA nanostructures, such as tetrahedra[112] and origami,[113–115] have the intrinsic ability to transport drug molecules into cancerous cells without the aid of transfection reagents. The loading efficiency and release kinetics of intercalating drugs can be modulated by designing DNA origami carriers with different degrees of global twist (Figure 4A).[113] Notably, DNA origami can enhance the cellular uptake and distribution of Dox and daunorubicin in Dox-resistant breast adenocarcinoma (MCF-7) cells[114] and daunorubicin-resistant leukemia (HL-60/ADR) cells,[115] respectively, resulting in a higher cytotoxicity than free drug at equal concentrations. Targeted chemotherapy was facilitated by targeting molecule-functionalized DNA nanocarriers ranging from aptamer-drug conjugates,[116] nanoflowers,[117,118] to DNA nanostructures (Figure 4B).[119–121] Moreover, drug release can be triggered by the degradation of carriers into nontoxic molecular components in response to a series of molecular stimuli or cancer hallmarks.[122–125]

Successful demonstration of improved specificity and efficacy *in vitro* lays the groundwork for transferring DNA-based drug delivery systems toward *in vivo* implementation. The efficacy of several DNA-based chemotherapeutic systems has been proven in mouse models. An aptamer-tethered DNA duplex chain (termed "nanotrain") was self-assembled via hybridization chain reaction (HCR), which specifically delivered Dox to human T cell acute lymphocytic leukemia (CEM) cells and slowed down tumor growth in a mouse xenograft model.[127] Due to a wide distribution in size, drugs delivered by this type of carriers are not homogeneous; i.e., each carrier

can deliver a different amount of drug molecules. Moreover, carriers of difference sizes may follow multiple endocytic pathways and result in potential side effects. The drug-loading ratio can be accurately controlled by covalently integrating drug molecules into DNA oligonucleotides that further self-assemble into shape-defined nanostructures. FU and CPT were incorporated into DNA strands composing DNA nanocages,[128,129] showing an improved efficacy in inhibiting the growth of HeLa and HCT166 tumors *in vivo*, respectively. Using DNA origami as drug-delivery vehicles was demonstrated *in vivo* by delivering Dox-loaded triangular DNA origami to nude mice bearing human orthotopic breast (MDA-MB-231) tumors (Figure 4C).[126] The triangular DNA origami passively accumulated in the tumor region due to the abnormal permeability of tumor vessels and the lack of effective lymphatic drainage, an effect called enhanced permeability and retention (EPR). Besides conventional drug-delivery strategy, which relies on the degradation of carriers and the passive release of the cargo in the endocytic pathway, various triggered-release mechanisms have been implemented with DNA-based carriers *in vivo*. A telomerase-responsive DNA icosahedron nanostructure was designed to encapsulate and deliver platinum nanodrugs into cisplatin-resistant human gastric carcinomas (BGC823/DDP) cells for *in vivo* tumor inhibition.[130] ATP-responsive nanogels,[123] H_2O_2-degradable DNA nanoflowers,[124] and self-catabolic DNAzyme nanosponges[125] were programmed to release Dox in response to ATP, H_2O_2, or an acidic microenvironment in order to inhibit human breast adenocarcinoma (MDA-MB-231), human colon cancer (HCT-166), and subcutaneous HeLa tumors, respectively. Most *in vivo* chemotherapeutic systems were conducted via systemic administration (e.g., intravenous injection). Report on the transdermal delivery of Dox with DNA nanostructures provides new insights into topical, noninvasive drug-delivery methods (Figure 4D).[95] The 17-nm DNA tetrahedron can deliver Dox to ~400 μm from the surface of mouse skin, inhibiting melanoma tumor growth with comparable efficacy to intratumoral injection and microneedle application of Dox.[95] Overall, DNA nanotechnology provides abundant structural and functional modules for improving the safety, specificity, and efficacy of chemotherapy. Combining chemotherapy with other therapeutic strategies could further enhance its therapeutic potency. Combination therapy will be discussed later in this section.

Gene therapy

Gene therapy can be achieved via the delivery of healthy genes or gene regulators into abnormal cells for disease prevention or treatment. DNA nanotechnology facilitates gene therapy via a nonviral delivery of gene regulators that include antisense DNA (ASD), DNAzyme, and small interfering RNA (siRNA) for post-transcriptional gene silencing. To prevent the translation of undesirable proteins, ASD, DNAzyme, and siRNA mediate the degradation of target messenger RNA (mRNA) via different mechanisms: ASD induces the hydrolysis of targeted mRNA by RNase H, DNAzyme recognizes and cleaves mRNA at a specific site, and siRNA induces mRNA degradation by forming the RNA-induced silencing complex (RISC). Poor cellular uptake of these nucleic-acid gene regulators is a major issue that limits efficient gene silencing. DNA nanotechnology provides various types of carriers, which can be easily loaded with nucleic-acid gene regulators and internalized via the endocytic pathway.

As compared with ASD alone, integrating ASD onto a DNA tetrahedron doubled its uptake and gene silencing effect (Figure 5A).[131] To increase the amount of cargos that can reach the cytoplasm via the endocytic pathway, carriers with a higher loading capacity are preferred for ASD delivery.[132,133] For example, multifunctional nanogels, which integrate ASD, DNAzyme, aptamer, and glutathione (GSH)-responsive disulfide linkage, can downregulate genes that are associated with the

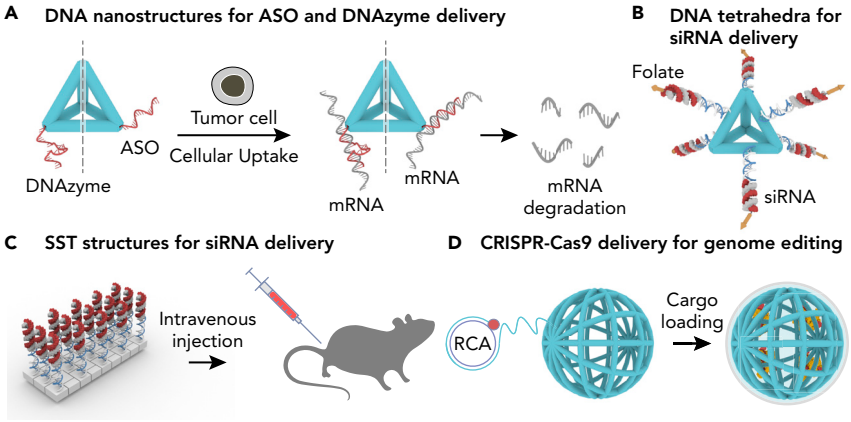

Figure 5. DNA nanotechnology for gene therapy

(A) Nonviral delivery of ASO and DNAzyme by a DNA tetrahedron for post-transcriptional gene silencing. DNA nanocarriers enhance the cellular uptake of ASO and DNAzyme. ASO and DNAzyme inhibit downstream protein production by degrading the corresponding mRNA.

(B) Folate-modified DNA tetrahedra were used for the targeted delivery of siRNA to KB xenograft tumors *in vivo*.[134] The spatial organization of folate on the DNA tetrahedra plays an important role in determining the efficiency of gene silencing. Reproduced with permission from Lee et al.[134] Copyright 2012 Springer Nature.

(C) siRNA delivery by SST carriers for Bcl2 depletion *in vivo*.[135] Efficient uptake of a small rectangular structure led to an effective inhibition of DMS53 tumor growth *in vivo*. Reproduced with permission from Rahman et al.[135] Copyright 2017 WILEY-VCH.

(D) CRISPR-Cas9 delivery by using PEI-coated DNA nanoclews for genome editing.[136] EGFP depletion was observed in ~25% of U2OS.EGFP cells after intratumoral injection. RCA, rolling circle amplification. Reproduced with permission from Sun et al.[136] Copyright 2012 WILEY-VCH.

proliferation of human lung adenocarcinoma epithelial (A549) cells in a targeted and stimulus-responsive manner.[133]

RNA interference (RNAi) has become a ubiquitous tool in biological research and a new frontier for therapeutics due to its structural simplicity, stealth administration, and universality.[137] siRNAs can be delivered by various DNA nanostructures and downregulate proteins such as survivin and Bcl2, which are involved in apoptosis inhibition of SKOV-3 and DMS53 cells, respectively.[85,135] Ideally, if the nucleic acid cargo can be directly delivered into the cytosol, the dose requirement could be greatly reduced. Covalently modifying siRNA or ASD with a disulfide group at its terminus[138] or packaging ASD with a guanidinium-disulfide monomer[86] can facilitate a transmembrane delivery to the cytoplasm within 30 min after administration. These strategies have the potential to be implemented into DNA nanostructures for ultrafast cytosolic delivery of cargos.

Most of the *in vivo* gene therapy experiments are based on RNAi. One of the biggest challenges for the implementation of gene therapy *in vivo* is maintaining the structural integrity of the therapeutic nucleic acids during delivery. DNA tetrahedra were initially used as a scaffold to organize siRNA and folate with well-defined location, density, and spatial orientation for targeted gene silencing of firefly-luciferase-expressing KB xenografts (Figure 5B).[134] The assembled carrier circulated 4 times longer in the blood than the siRNA alone and resulted in a 60% reduction in the bioluminescent intensity without a detectable immune response. Microsponges[139] and nanogels[140] are also capable of siRNA delivery for the effective knockdown of firefly luciferase in T22-Luc tumor-bearing mice and polo-like kinase 1 (PLK1) expression in MDA-MB-231 tumor-bearing mice, respectively. In these particles, DNA mainly serves as an RNA transcription template

or siRNA anchoring point. In the first report of using SST nanocarriers for therapeutics, a set of rectangular and tubular SST nanostructures were screened to deliver siRNA for the depletion of an antiapoptotic protein, Bcl2 (Figure 5C).[135] Efficient uptake of the smallest rectangular structure led to a 90% knockdown of the Bcl2 protein *in vitro* and an effective inhibition of DMS53 tumor growth *in vivo*. An intriguing example of genome editing was demonstrated by the intratumoral injection of Cas9/sgRNA-loaded DNA nanoclews for targeted enhanced green fluorescent protein (EGFP) disruption (Figure 5D).[136] The successful delivery of CRISPR-Cas9 by DNA nanocarriers suggested new opportunities for integrating molecular-biology techniques with the DNA nanotechnology platform. Smart carriers with specific targeting and triggered release functions are in high demand for gene therapy and combination therapy.

Phototherapy

Photodynamic therapy (PDT) and photothermal therapy (PTT) are the two major forms of phototherapies that have been advanced by DNA nanotechnology. DNA carriers improve the solubility, specificity, and cellular uptake of photosensitizers and photothermal materials, and promote reactive oxygen species (ROS) or heat generation upon irradiation to damage malignant cells. Compared with chemotherapy, phototherapy is less likely to cause drug resistance, long-term toxicity, and side effects.

Aptamer-photosensitizer complexes such as aptamer-chlorin e6 (Ce6) conjugates[141] and porphyrin photosensitizer (TMPyP4)-loaded aptamers[142] were the earliest constructs for targeted PDT. Aptamers confine photosensitizers to malignant cells and minimize the indiscriminate damage of ROS on healthy cells. The PDT effect can be locally amplified with the use of a catalytic DNA circuit, where the catalyst-sequence-bearing aptamer activates and catalyzes the revelation of Ce6 on the cell surface.[143] Moreover, different aptamers can be multiplexed in a single circuit for logic-based cell recognition and PDT.[144,145] For example, a 3-arm DNA "nanoclaw" carrying an sgc8c aptamer, a TC01 aptamer, and a Ce6 photosensitizer on each arm could operate based on the AND logic gate and activate Ce6 only when both aptamers recognized targeted surface markers on the same cell (Figure 6A).[144] DNA origami can improve the solubility and photostability of BMEPC, a carbazole-derived photosensitizer, and enhance radical production in MCF-7 cells (Figure 6B).[146] Photothermal materials are usually bulkier and less programmable than photosensitizers; therefore, among all types of DNA nanostructures, DNA origami is usually used as the carrier for PTT.[147–149]

Compared with chemo- and gene therapy, there are not as many applications of DNA nanotechnology-based phototherapy *in vivo*. DNA nanosponges were loaded with sgc8c aptamer, catalase, and TMPyP4 photosensitizer to achieve effective PDT under hypoxic conditions (Figure 6C).[150] The sensitization of PDT could be enhanced by including the antisense sequence-targeting hypoxia-inducible factor 1α (HIF-1α), a major transcription factor associated with tumor hypoxia.[150] The passive tumor-targeting property of DNA origami has been used to assist the delivery of gold nanorods (AuNRs) to the tumor site for a more efficient elevation of local temperatures upon near-infrared (NIR) irradiation and for better PTT efficacy than AuNR itself (Figure 6D).[147,148] Integrating imaging agents into these therapeutic systems enabled a real-time visualization of their biodistribution and tumor uptake via fluorescence or optoacoustic imaging.[148,150] In summary, DNA nanotechnology primarily enhances the specificity and efficacy of phototherapy. Combining phototherapy with other therapeutic strategies could result in a more synergistic effect and light-controlled drug release.

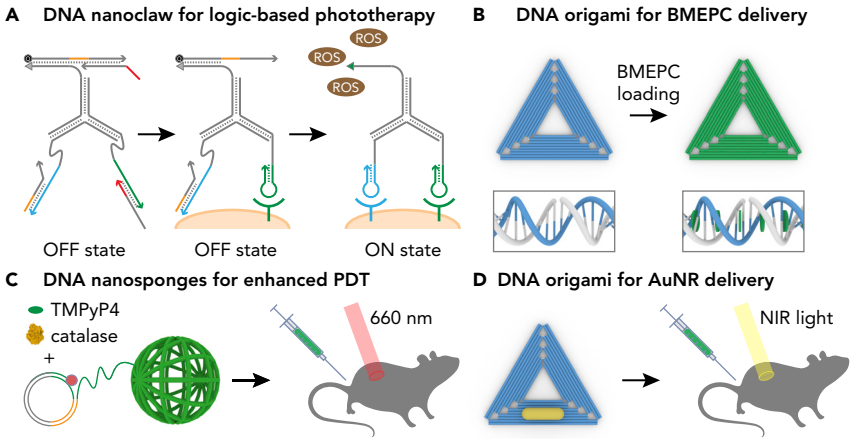

Figure 6. DNA nanotechnology for phototherapy

(A) A logic-based DNA nanoclaw for cancer targeting and PDT.[144] The photosensitizer (green triangle) is activated only when both targeting domains are attached to the surface biomarkers on cancer cells. Reproduced with permission from You et al.[144] Copyright 2014 American Chemical Society.

(B) DNA origami for photosensitizer delivery into tumor cells.[146] The model photosensitizer, BMEPC, can be loaded onto DNA origami by intercalation. DNA origami reduces the photobleaching of BMEPC and enhances its radical production. Reproduced with permission from Zhuang et al.[146] Copyright 2016 American Chemical Society.

(C) DNA nanosponges for enhanced PDT.[150] Co-delivery of TMPyP4 and catalase by DNA nanosponges can relieve hypoxia-associated resistance and enhance the efficacy of TMPyP4. Reproduced with permission from Pan et al.[150] Copyright 2019 WILEY-VCH.

(D) AuNR delivery by DNA origami.[148] Optoacoustic imaging and photothermal therapy are simultaneously achieved *in vivo*. Reproduced with permission from Du et al.[148] Copyright 2016 WILEY-VCH.

Immunotherapy

Unmethylated cytosine-phosphate-guanine (CpG) oligonucleotides with certain sequence motifs can be recognized by toll-like receptor 9 (TLR9) and stimulate cytokine release and an immune response.[151] TLR9 is expressed in antigen-presenting cells (APCs) and is generally located in the endosome. Since DNA nanostructures are usually internalized into APCs via the endocytic pathway, the delivery of synthetic CpG oligonucleotides by DNA nanostructures is a convenient method for immune stimulation and immunotherapy.

Cellular delivery of well-studied synthetic CpG motifs has been achieved with a variety of DNA nanocarriers, including Y-shaped tiles,[152] dendrimers,[153] hydrogels,[154] DNA tetrahedra,[155] and DNA origami tubes[156] (Figure 7A). As indicated by enzyme-linked immunosorbent assay (ELISA), compared with CpG motif itself, a significantly higher level of cytokines (e.g., tumor necrosis factor (TNF)-α, interleukin (IL)-6, and IL-12) can be induced by the CpG motif delivered by DNA nanocarriers.[152–156] The cytokine levels were positively correlated with the number of CpG motifs displayed on a single DNA tetrahedron.[155] The activation of immune cells induced by DNA origami tubes carrying 62 CpG sequences was also indicated by a greater amount of the early activation marker CD69 on dendritic and B cells.[156]

A synthetic vaccine was rationally designed by precisely assembling CpG adjuvants and a model antigen, streptavidin (STV), onto DNA tetrahedra (Figure 7A).[157] The vaccine mimicked a natural viral particle, inducing an antibody response against STV and maintaining a higher anti-STV IgG level than that of free CpG and STV for

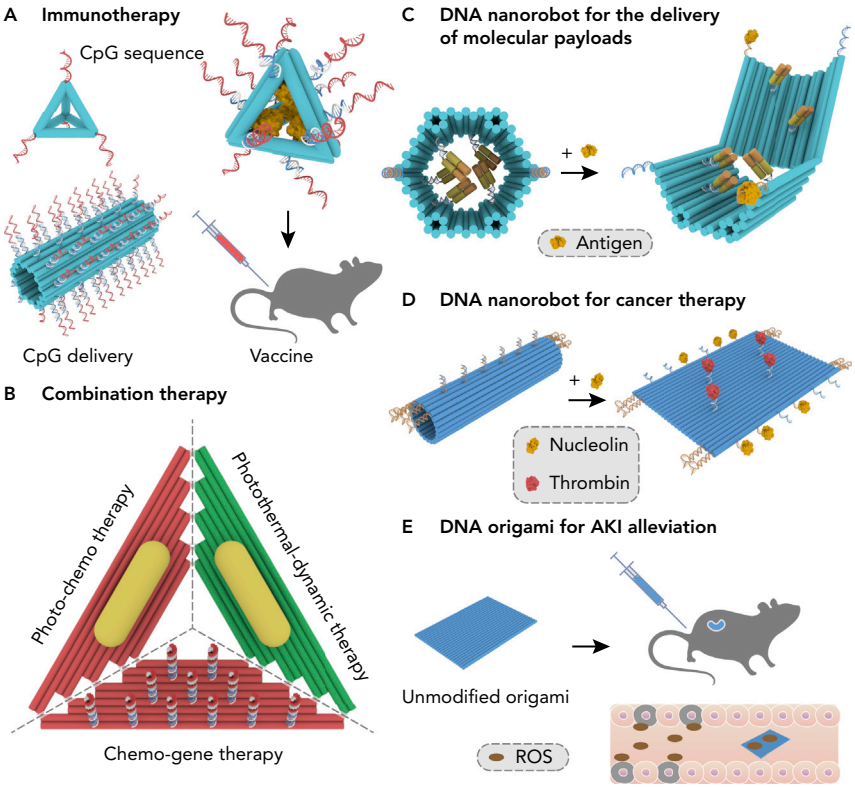

Figure 7. Other promising therapeutic strategies facilitated by DNA nanotechnology

(A) Two strategies of using DNA nanostructures for immunotherapy. DNA nanostructures enhance the cellular uptake of immunostimulatory CpG sequences and induce a high-level secretion of various pro-inflammatory cytokines.[155,156] Co-delivery of CpG adjuvants and a model antigen by DNA tetrahedra induces strong and long-lasting antibody responses against the model antigen.[157] (Top left) Reproduced with permission from Li et al.[155] Copyright 2011 American Chemical Society. (Bottom left) Reproduced with permission from Schuller et al.[156] Copyright 2011 American Chemical Society. (Right) Reproduced with permission from Liu et al.[157] Copyright 2012 American Chemical Society.

(B) Three common strategies for combination therapy. Combination therapy can be achieved by the co-delivery of two synergistic therapeutic agents by the same DNA carrier. Examples of combination therapy include photo-chemo therapy, chemo-gene therapy, and photothermal-dynamic therapy.

(C) A prototype DNA nanorobot for triggered cargo exposure based on AND logic.[158] The sequestered antibody fragments are exposed for cell signaling regulation when both aptamer-based locks are attached to the corresponding cell surface markers. Reproduced with permission from Douglas et al.[158] Copyright 2012 AAAS.

(D) A nucleolin-responsive DNA nanorobot for tumor inhibition *in vivo*.[159] The encapsulated blood coagulation protease, thrombin, is exposed in response to nucleolin and induces intravascular thrombosis to inhibit tumor growth. Reproduced with permission from Li et al.[159] Copyright 2018 Springer Nature.

(E) DNA origami with preferred renal uptake and AKI alleviation effect.[93] Compared with DNA origami with other geometries, rectangular DNA origami showed an outstanding ROS-scavenging effect, which was further used for AKI treatment. Reproduced with permission from Jiang et al.[93] Copyright 2018 Springer Nature.

over 70 days. To prevent postsurgical tumor relapse, an anti-PD-1 antibody was encapsulated in a CpG-encoded nano-cocoon and locally injected into the resection bed.[160] A local proinflammatory environment triggered the sustained release of CpG oligonucleotides and anti-PD-1 antibodies, inducing an antitumor effect that was effective for both remaining and metastatic tumors. Overall, the use of DNA nanotechnology for immunotherapy mainly focuses on CpG delivery. Recently,

aptamer-equipping strategies for immune-cell engineering started to emerge,[161,162] which hold great potential in adoptive immunotherapy.

Combination therapy

Therapeutic agents kill malignant cells with chemical toxicity, biological interference, or physical damage. However, intrinsic limitations and adverse effects could still cause a simple therapeutic strategy to fail. For example, tumor cells could potentially develop resistance to chemotherapy. Phototherapy has limited tissue-penetration depth and limited efficacy on metastatic cancer. Gene therapy is usually short lived and might incite an immune response. To address these issues, DNA nanotechnology provides a multifunctional platform that integrates therapeutic strategies into a single formulation to promote a synergistic effect of different strategies (Figure 7B). Moreover, the dose and ratio of therapeutic agents can be finely tuned on the DNA platform, minimizing dose-dependent toxicity and other side effects without compromising efficacy.

Combination therapy has been proven to be effective against MDR cancer cells. For example, Dox and the gene encoding tumor suppressor p53 were delivered into MDR cancer (MCF-7R) cells by a triangular DNA origami to inhibit tumor growth without eliciting apparent systemic toxicity or immune response.[163] Dox, AuNR, and MUC1 aptamer have been combined on a triangular DNA origami for targeted photo-chemo therapy *in vitro*.[149] The photothermal effects of AuNR upon NIR irradiation inhibited the expression of glycoprotein (P-gp), a typical drug efflux pump involved in drug resistance, and enhanced the efficacy of Dox on Dox-resistant MCF-7/ADR cells.[149] Another widely used formulation is the co-delivery of chemotherapy drug along with gene regulators targeting P-gp. The cytotoxic effect of Dox on MDR tumors can be effectively enhanced by various P-gp inhibitors, including ASD nanoparticles[164] and small-hairpin RNA (shRNA) transcription templates.[165] Combination therapy explores the complementary and synergistic effects among a combination of therapeutic strategies, providing potential solutions for tumor types that are difficult to treat with a single therapy.

Therapeutic DNA nanorobots

Therapeutic DNA nanorobots are DNA devices that can automatically perform therapeutic tasks, including target recognition, drug delivery, and triggered release, following pre-encoded rules. For example, a prototype DNA nanorobot was programmed to open and expose its payloads in response to a combination of "key" antigens that were present on the surface of a cell (Figure 7C).[158] By varying its payloads, the nanorobot could carry out user-defined tasks such as cell discrimination, growth arrest of aggressive natural killer leukemia (NKL) cells, and T cell activation. Recently, therapeutic DNA nanorobot was successfully applied to cancer therapy *in vivo* (Figure 7D).[159] Rather than being internalized into tumor cells, the nanorobot was activated in response to nucleolin in the tumor vessel and subsequently exposed thrombins that were initially sequestered to trigger intravascular thrombosis at the tumor site and induced tumor necrosis. No significant changes in the blood coagulation parameters, cytokine levels, or cytotoxicity were observed when testing the nanorobot in healthy mice and Bama miniature pigs, suggesting that their use in the normal tissues of large animals would be considered safe.[159]

A CASE STUDY FOR TREATING KIDNEY DISEASES

The kidney is an important organ that filters blood, removes wastes and toxins, maintains the balance of body fluids and electrolytes, and helps control blood pressure in animal bodies. Kidney dysfunction can lead to serious or even life-threatening health issues. Several recent studies that highlight the potential of applying DNA nanotechnology to the treatment of kidney diseases (or nephropathy) are discussed here.

Renal uptake of DNA nanostructures

There are various biological interfaces in living bodies, which form the barriers for drug delivery and affect the organ distribution of nanoparticles.[166] Given the extraordinary programmability of DNA nanostructures, one may envision that their abilities to pass through biological barriers and target specific organs (e.g., brain, liver, kidney, etc.) can be improved by simply tuning their physical properties. In general, nanoparticles ranging from 30 to 150 nm are not easily cleared by kidney filtration, while those with a small hydrodynamic size (5–7 nm) or with high aspect ratios and small diameters can be cleared as they fall below the filtration threshold of kidney.[167] Taking advantage of this effect, DNA nanostructures can be designed to have distinct renal uptake and retention profiles. For example, the renal clearance of Cu^{64}-labeled small DNA tetrahedra (~7 nm) can be monitored for the evaluation of kidney functions via positron emission tomography (PET) imaging.[94] In another study, PET imaging of the biodistribution of Cu^{64}-labeled DNA origami nanostructures revealed that intact structures preferentially accumulate in kidneys within 12 h and showed whole-body clearance after 24 h postinjection.[93] In contrast, the scaffold strand and partially folded DNA origami nanostructures were primarily sequestered in the liver and cleared by the mononuclear phagocyte system.[93] Thus, structural compactness and intact folding are two factors that reduce hepatic sequestration and improve kidney accumulation of DNA nanostructures.

Treating AKI with DNA nanostructures

Kidney diseases can be generally categorized into acute kidney injury (AKI), chronic kidney disease (CKD), and kidney-related diseases (e.g., kidney tumor). A rectangular DNA origami was exploited to alleviate rhabdomyolysis-induced AKI and protect renal cells from nephrotoxic agents (Figure 7E).[93] Its therapeutic effect was evaluated by dynamic PET imaging with ^{68}Ga-EDTA, blood tests, and kidney-tissue staining, which was similar to antioxidant N-acetylcysteine, a clinical drug for contrast-induced AKI prevention.[93]

Current research suggests the preferential renal uptake and the possibility of tuning the retention of DNA nanostructures in kidneys. Therefore, it is possible to apply DNA-based therapeutic formulations that have been proven effective *in vivo* to the treatments of other kidney diseases. The key is to find target molecules that are involved in the onset of kidney diseases. In addition to ROS, potential targets include noncoding RNAs,[168] and proteins such as kidney injury molecule-1 (KIM-1),[169] clusterin[170] and transforming growth factor-β (TGF-β).[171] Based on these targets, it is possible to design proper kidney-targeted DNA drug delivery systems for the treatment of other kidney diseases.

CHALLENGES AND PERSPECTIVES

In the past decade, remarkable progress has been made in structural DNA nanotechnology, which facilitates the use of designer DNA nanostructures for therapeutic applications. Complex higher-order DNA assemblies can be rationally and automatically designed and efficiently scaled-up. Chemical methods are developed to enhance the stability of DNA nanostructures in physiological environments. Synthesis and purification costs of DNA origami structures are expected to be greatly reduced by the recently reported mass-production and purification protocols. The modularity and programmability of the DNA platform allow various mechanisms of target recognition and triggered release to be implemented *in vivo*.

Several challenges still remain to be addressed before using DNA nanocarriers in clinical practice. Firstly, the structural heterogeneity of DNA nanocarriers may cause side effects. There is evidence that partially formed and intact DNA nanostructures have different preferences for organ accumulation.[93] It can be inferred that the organ-targeting ability of a DNA nanocarrier changes during circulation due to its gradual denaturation. Aberrant accumulation not only increases the burdens of healthy organs but also reduces the effective concentration of the drug at pathogenic sites. Moreover, precise dosing is challenging to achieve, especially for intercalating drugs, whose spectrum properties and loading and release profiles are affected by a number of factors.[13,172] In this respect, a gold standard is needed to enable cross-comparison between studies. Drug-loading capacity can be better controlled by covalently conjugating drug molecules onto DNA strands that compose a nanocarrier.[116,128,129] Biotechnological methods for the economical synthesis of drug-conjugated DNA rely on the enzymes that can catalyze the polymerization of nucleotide-containing unnatural base analogs. Although there are *in vitro* strategies available, *in vivo* production is largely elusive.

Secondly, changes in the surface chemistry of DNA nanocarriers may affect their biodistribution. For example, surface coating via electrostatic interaction is likely to change the circulating time, organ distribution, and toxicity of a DNA nanostructure. The resultant effects need to be characterized *in vivo*. Protein-corona formation is another factor that could drastically alter the surface chemistry of DNA nanostructures *in vivo*. Serum proteins can adhere to DNA nanostructures in both sequence-dependent and nonspecific manners, and further induce aggregation or promote clearance. To solve this problem, chemical modification and coating could effectively change the surface chemistry of DNA nanostructures and prevent protein-corona formation by reducing the affinity of serum proteins. Alternatively, the sequence of DNA nanostructures can be rationally designed to alter the profile of protein adsorption and facilitate organ targeting.

Moreover, the endocytosis mechanism of DNA nanostructures is not comprehensively understood. Although DNA nanotechnology has the unique advantage of designing arbitrary shapes with nanometer precision, the types of DNA nanostructures that have been tested for endocytosis are relatively limited. Comprehensive mechanistic studies that take structure characteristics, surface chemistry, and cell type into account are needed to predict the endocytosis and cellular fate of a specific construct. Getting trapped and degraded in the lysosome is usually the fate of internalized DNA nanostructures. Therefore, simple and robust strategies to facilitate endo/lysosomal escape, organelle targeting, and nuclear entry are in demand. Exploring nonendocytic pathway for DNA nanostructures to circumvent the acidic environment in the lysosome will greatly enhance the efficacy of therapeutic agents that function in the cytosol and nucleus.

Given the complex environments *in vivo*, the pharmacokinetics and pharmacodynamics of DNA nanostructures are complicated by many factors. Tracing the absorption, distribution, metabolism, and excretion of a DNA carrier and its cargo, as well as their effects on living organisms, are the key to evaluate dosing, efficacy, and adverse effects. Rapid clearance is a major obstacle that limits the efficacy of DNA-based drug-delivery systems at the current stage of research. A higher cost and toxicity will be associated with the repetitive administration of drugs at a higher dose to prolong the retention time.

Finally, the biosafety of DNA-based therapeutic systems should be evaluated in the long term. For example, the contamination of immunostimulatory endotoxin, which is involved in the preparation and amplification of DNA from Gram-negative bacteria, must be evaluated and reduced to the safe level. There is no indication of chronic toxicity induced by

DNA nanostructures so far, which can be largely attributed to the rapid clearance of DNA nanostructures from the body. Extending the retention of DNA nanostructure might bring new safety concerns. For example, given the complexity and randomness of DNA sequences in a nanostructure, extraneous DNA could randomly interfere with cellular RNA and induce other potential chronic toxicity. Moreover, the immune response has not been well characterized for DNA nanostructures *in vivo*. Although many studies report that CpG-free DNA nanostructures have minimal immunogenicity, immunostimulatory activity via TLR9-independent pathway is not negligible.[156] Principles to design DNA carriers that are compatible with the immune system of higher organisms are in need.[173]

Recent advances in DNA nanotechnology-based therapeutics have brought up new opportunities for future research. First, to obtain a general and comprehensive understanding of the endocytosis mechanism and the pharmacokinetics and pharmacodynamics of DNA nanostructures, it is necessary to summarize individual therapeutic examples into networks in order to better predict the interaction between DNA nanostructures and living organisms. Big data analytics and machine learning could help with the exploration of the vast design space for this purpose. Second, more diverse drug-administration routes are in demand. The successful implementation of transdermal Dox delivery[95] suggests the possibilities of developing other noninvasive drug-delivery routes such as intranasal administration. Topical administration via mucosa is expected to yield rapid drug absorption and reduced risk of systemic side effects, and may serve as a supplementary of radiation therapy for nasopharyngeal carcinoma and other diseases. Third, the ability to pattern diverse molecules with nanometer precision would facilitate the rational design of antivirals or vaccines. For example, a practical strategy to engineer antivirals is to block and deactivate viruses with patterned ligands that are templated by a DNA scaffold.[174] Another example is the effective B cell receptor activation *in vitro* by site-specifically displaying immunogens on a DNA template.[175] Implementation of these two systems *in vivo* remains to be explored. Fourth, DNA nanostructure has the potential to be used as an immunosuppressant to regulate the excessive release of cytokines. This is helpful for alleviating cytokine storm, which is a catastrophic immune problem that may cause permanent organ damage or even death. At last, replicable single-stranded DNA origami structures are promising candidates for therapeutics as they have suitable size, addressability, and exceptional thermostability. DNA nanotechnology-based therapeutics will certainly benefit from the development of new drugs, therapeutic strategies, and formulations. Solutions to intractable solid tumors and vascularized metastases will be promising to pursue. As a rapidly evolving research direction, DNA nanotechnology-based therapeutics would serve as a smart, custom platform for clinical therapeutics.

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AUTHOR CONTRIBUTIONS

S.J., Z.G., and S.M. investigated the literature and wrote and revised the manuscript. C.F. and H.Y. supervised the writing of the manuscript and revised the manuscript.

REFERENCES

1. Kim, B.Y., Rutka, J.T., and Chan, W.C. (2010). Nanomedicine. N. Engl. J. Med. *363*, 2434–2443.
2. Wagner, V., Dullaart, A., Bock, A.K., and Zweck, A. (2006). The emerging nanomedicine landscape. Nat. Biotechnol. *24*, 1211–1217.
3. Moghimi, S.M., Hunter, A.C., and Murray, J.C. (2005). Nanomedicine: current status and future prospects. FASEB J *19*, 311–330.
4. Bobo, D., Robinson, K.J., Islam, J., Thurecht, K.J., and Corrie, S.R. (2016). Nanoparticle-based medicines: a review of FDA-approved materials and clinical trials to date. Pharm. Res. *33*, 2373–2387.
5. Seeman, N.C. (1982). Nucleic acid junctions and lattices. J. Theor. Biol. *99*, 237–247.
6. Seeman, N.C. (2003). DNA in a material world. Nature *421*, 427–431.
7. Pinheiro, A.V., Han, D., Shih, W.M., and Yan, H. (2011). Challenges and opportunities for structural DNA nanotechnology. Nat. Nanotechnol. *6*, 763–772.
8. Zhang, F., Nangreave, J., Liu, Y., and Yan, H. (2014). Structural DNA nanotechnology: state of the art and future perspective. J. Am. Chem. Soc. *136*, 11198–11211.
9. Seeman, N.C., and Sleiman, H.F. (2018). DNA nanotechnology. Nat. Rev. Mater. *3*, 17068.
10. Li, J., Fan, C., Pei, H., Shi, J., and Huang, Q. (2013). Smart drug delivery nanocarriers with self-assembled DNA nanostructures. Adv. Mater. *25*, 4386–4396.
11. Chen, Y.J., Groves, B., Muscat, R.A., and Seelig, G. (2015). DNA nanotechnology from the test tube to the cell. Nat. Nanotechnol. *10*, 748–760.
12. Hu, Q., Li, H., Wang, L., Gu, H., and Fan, C. (2019). DNA nanotechnology-enabled drug delivery systems. Chem. Rev. *119*, 6459–6506.
13. Keller, A., and Linko, V. (2020). Challenges and perspectives of DNA nanostructures in biomedicine. Angew Chem Int Ed Engl *59*, 15818–15833.
14. Chen, J.H., and Seeman, N.C. (1991). Synthesis from DNA of a molecule with the connectivity of a cube. Nature *350*, 631–633.
15. Yan, H., Park, S.H., Finkelstein, G., Reif, J.H., and LaBean, T.H. (2003). DNA-templated self-assembly of protein arrays and highly conductive nanowires. Science *301*, 1882–1884.
16. Rothemund, P.W. (2006). Folding DNA to create nanoscale shapes and patterns. Nature *440*, 297–302.
17. Castro, C.E., Kilchherr, F., Kim, D.N., Shiao, E.L., Wauer, T., Wortmann, P., Bathe, M., and Dietz, H. (2011). A primer to scaffolded DNA origami. Nat. Methods *8*, 221–229.
18. Wei, B., Dai, M., and Yin, P. (2012). Complex shapes self-assembled from single-stranded DNA tiles. Nature *485*, 623–626.
19. Ke, Y., Ong, L.L., Shih, W.M., and Yin, P. (2012). Three-dimensional structures self-assembled from DNA bricks. Science *338*, 1177–1183.
20. Han, D., Qi, X., Myhrvold, C., Wang, B., Dai, M., Jiang, S., Bates, M., Liu, Y., An, B., Zhang, F., et al. (2017). Single-stranded DNA and RNA origami. Science *358*, eaao2648.
21. Kallenbach, N.R., Ma, R.I., and Seeman, N.C. (1983). An immobile nucleic acid junction constructed from oligonucleotides. Nature *305*, 829–831.
22. Goodman, R.P., Berry, R.M., and Turberfield, A.J. (2004). The single-step synthesis of a DNA tetrahedron. Chem. Commun. (Camb.) *2004*, 1372–1373.
23. Goodman, R.P., Schaap, I.A., Tardin, C.F., Erben, C.M., Berry, R.M., Schmidt, C.F., and Turberfield, A.J. (2005). Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. Science *310*, 1661–1665.
24. Fu, T.J., and Seeman, N.C. (1993). DNA double-crossover molecules. Biochemistry *32*, 3211–3220.
25. He, Y., Ye, T., Su, M., Zhang, C., Ribbe, A.E., Jiang, W., and Mao, C. (2008). Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra. Nature *452*, 198–201.
26. Zhang, C., Su, M., He, Y., Zhao, X., Fang, P.A., Ribbe, A.E., Jiang, W., and Mao, C. (2008). Conformational flexibility facilitates self-assembly of complex DNA nanostructures. Proc. Natl. Acad. Sci. USA *105*, 10665–10669.
27. Rothemund, P.W., Ekani-Nkodo, A., Papadakis, N., Kumar, A., Fygenson, D.K., and Winfree, E. (2004). Design and characterization of programmable DNA nanotubes. J. Am. Chem. Soc. *126*, 16344–16352.
28. Winfree, E., Liu, F., Wenzler, L.A., and Seeman, N.C. (1998). Design and self-assembly of two-dimensional DNA crystals. Nature *394*, 539–544.
29. He, Y., Chen, Y., Liu, H., Ribbe, A.E., and Mao, C. (2005). Self-assembly of hexagonal DNA two-dimensional (2D) arrays. J. Am. Chem. Soc. *127*, 12202–12203.
30. He, Y., Tian, Y., Ribbe, A.E., and Mao, C. (2006). Highly connected two-dimensional crystals of DNA six-point-stars. J. Am. Chem. Soc. *128*, 15978–15979.
31. Liu, L., Li, Z., Li, Y., and Mao, C. (2019). Rational design and self-assembly of two-dimensional, dodecagonal DNA quasicrystals. J. Am. Chem. Soc. *141*, 4248–4251.
32. Zheng, J., Birktoft, J.J., Chen, Y., Wang, T., Sha, R., Constantinou, P.E., Ginell, S.L., Mao, C., and Seeman, N.C. (2009). From molecular to macroscopic via the rational design of a self-assembled 3D DNA crystal. Nature *461*, 74–77.
33. Douglas, S.M., Dietz, H., Liedl, T., Högberg, B., Graf, F., and Shih, W.M. (2009). Self-assembly of DNA into nanoscale three-dimensional shapes. Nature *459*, 414–418.
34. Ke, Y., Douglas, S.M., Liu, M., Sharma, J., Cheng, A., Leung, A., Liu, Y., Shih, W.M., and Yan, H. (2009). Multilayer DNA origami packed on a square lattice. J. Am. Chem. Soc. *131*, 15903–15908.
35. Ke, Y., Voigt, N.V., Gothelf, K.V., and Shih, W.M. (2012). Multilayer DNA origami packed on hexagonal and hybrid lattices. J. Am. Chem. Soc. *134*, 1770–1774.
36. Dietz, H., Douglas, S.M., and Shih, W.M. (2009). Folding DNA into twisted and curved nanoscale shapes. Science *325*, 725–730.
37. Han, D., Pal, S., Nangreave, J., Deng, Z., Liu, Y., and Yan, H. (2011). DNA origami with complex curvatures in three-dimensional space. Science *332*, 342–346.
38. Yin, P., Hariadi, R.F., Sahu, S., Choi, H.M., Park, S.H., Labean, T.H., and Reif, J.H. (2008). Programming DNA tube circumferences. Science *321*, 824–826.
39. Ke, Y., Ong, L.L., Sun, W., Song, J., Dong, M., Shih, W.M., and Yin, P. (2014). DNA brick crystals with prescribed depths. Nat. Chem. *6*, 994–1002.
40. Han, D., Pal, S., Yang, Y., Jiang, S., Nangreave, J., Liu, Y., and Yan, H. (2013). DNA gridiron nanostructures based on four-arm junctions. Science *339*, 1412–1415.
41. Benson, E., Mohammed, A., Gardell, J., Masich, S., Czeizler, E., Orponen, P., and Högberg, B. (2015). DNA rendering of polyhedral meshes at the nanoscale. Nature *523*, 441–444.
42. Zhang, F., Jiang, S., Wu, S., Li, Y., Mao, C., Liu, Y., and Yan, H. (2015). Complex wireframe DNA origami nanostructures with multi-arm junction vertices. Nat. Nanotechnol. *10*, 779–784.
43. Veneziano, R., Ratanalert, S., Zhang, K., Zhang, F., Yan, H., Chiu, W., and Bathe, M. (2016). Designer nanoscale DNA assemblies programmed from the top down. Science *352*, 1534.
44. Shih, W.M., Quispe, J.D., and Joyce, G.F. (2004). A 1.7-kilobase single-stranded DNA that folds into a nanoscale octahedron. Nature *427*, 618–621.
45. Qi, X., Zhang, F., Su, Z., Jiang, S., Han, D., Ding, B., Liu, Y., Chiu, W., Yin, P., and Yan, H. (2018). Programming molecular topologies from single-stranded nucleic acids. Nat. Commun. *9*, 4579.
46. Tikhomirov, G., Petersen, P., and Qian, L. (2017). Programmable disorder in random DNA tilings. Nat. Nanotechnol. *12*, 251–259.
47. Iinuma, R., Ke, Y., Jungmann, R., Schlichthaerle, T., Woehrstein, J.B., and Yin, P. (2014). Polyhedra self-assembled from DNA tripods and characterized with 3D DNA-PAINT. Science *344*, 65–69.
48. Gerling, T., Wagenbauer, K.F., Neuner, A.M., and Dietz, H. (2015). Dynamic DNA devices and assemblies formed by shape-complementary, non-base pairing 3D components. Science *347*, 1446–1452.

49. Wagenbauer, K.F., Sigl, C., and Dietz, H. (2017). Gigadalton-scale shape-programmable DNA assemblies. Nature *552*, 78–83.

50. Tikhomirov, G., Petersen, P., and Qian, L. (2017). Fractal assembly of micrometre-scale DNA origami arrays with arbitrary patterns. Nature *552*, 67–71.

51. Ong, L.L., Hanikel, N., Yaghi, O.K., Grun, C., Strauss, M.T., Bron, P., Lai-Kee-Him, J., Schueder, F., Wang, B., Wang, P., et al. (2017). Programmable self-assembly of three-dimensional nanostructures from 10,000 unique components. Nature *552*, 72–77.

52. Cutler, J.I., Auyeung, E., and Mirkin, C.A. (2012). Spherical nucleic acids. J. Am. Chem. Soc. *134*, 1376–1391.

53. Barnaby, S.N., Sita, T.L., Petrosko, S.H., Stegh, A.H., and Mirkin, C.A. (2015). Therapeutic applications of spherical nucleic acids. Cancer Treat. Res. *166*, 23–50.

54. Sobczak, J.P., Martin, T.G., Gerling, T., and Dietz, H. (2012). Rapid folding of DNA into nanoscale shapes at constant temperature. Science *338*, 1458–1461.

55. Yurke, B., Turberfield, A.J., Mills, A.P., Jr., Simmel, F.C., and Neumann, J.L. (2000). A DNA-fuelled molecular machine made of DNA. Nature *406*, 605–608.

56. Andersen, E.S., Dong, M., Nielsen, M.M., Jahn, K., Subramani, R., Mamdouh, W., Golas, M.M., Sander, B., Stark, H., and Oliveira, C.L.P. (2009). Self-assembly of a nanoscale DNA box with a controllable lid. Nature *459*, 73–76.

57. Zhang, D.Y., and Seelig, G. (2011). Dynamic DNA nanotechnology using strand-displacement reactions. Nat. Chem. *3*, 103–113.

58. Xin, Y., Kielar, C., Zhu, S., Sikeler, C., Xu, X., Möser, C., Grundmeier, G., Liedl, T., Heuer-Jungemann, A., Smith, D.M., and Keller, A. (2020). Cryopreservation of DNA origami nanostructures. Small *16*, e1905959.

59. Hahn, J., Wickham, S.F., Shih, W.M., and Perrault, S.D. (2014). Addressing the instability of DNA nanostructures in tissue culture. ACS Nano *8*, 8765–8775.

60. Martin, T.G., and Dietz, H. (2012). Magnesium-free self-assembly of multi-layer DNA objects. Nat. Commun. *3*, 1103.

61. Kielar, C., Xin, Y., Shen, B., Kostiainen, M.A., Grundmeier, G., Linko, V., and Keller, A. (2018). On the stability of DNA origami nanostructures in low-magnesium buffers. Angew. Chem. Int. Ed. *57*, 9470–9474.

62. Chandrasekaran, A.R., Vilcapoma, J., Dey, P., Wong-Deyrup, S.W., Dey, B.K., and Halvorsen, K. (2020). Exceptional nuclease resistance of paranemic crossover (PX) DNA and crossover-dependent biostability of DNA motifs. J. Am. Chem. Soc. *142*, 6814–6821.

63. Ramakrishnan, S., Shen, B., Kostiainen, M.A., Grundmeier, G., Keller, A., and Linko, V. (2019). Real-time observation of superstructure-dependent DNA origami digestion by DNase I using high-speed atomic force microscopy. Chembiochem *20*, 2818–2823.

64. Suma, A., Stopar, A., Nicholson, A.W., Castronovo, M., and Carnevale, V. (2020). Global and local mechanical properties control endonuclease reactivity of a DNA origami nanostructure. Nucleic Acids Res *48*, 4672–4680.

65. Khvorova, A., and Watts, J.K. (2017). The chemical evolution of oligonucleotide therapies of clinical utility. Nat. Biotechnol. *35*, 238–248.

66. Kim, K.R., Kim, H.Y., Lee, Y.D., Ha, J.S., Kang, J.H., Jeong, H., Bang, D., Ko, Y.T., Kim, S., Lee, H., and Ahn, D.R. (2016). Self-assembled mirror DNA nanostructures for tumor-specific delivery of anticancer drugs. J. Control. Release *243*, 121–131.

67. Keum, J.W., and Bermudez, H. (2009). Enhanced resistance of DNA nanostructures to enzymatic digestion. Chem. Commun. (Camb.) *7*, 7036–7038.

68. Cassinelli, V., Oberleitner, B., Sobotta, J., Nickels, P., Grossi, G., Kempter, S., Frischmuth, T., Liedl, T., and Manetto, A. (2015). One-step formation of "chain-armor"-stabilized DNA nanostructures. Angew. Chem. Int. Ed. Engl. *54*, 7795–7798.

69. Gerling, T., Kube, M., Kick, B., and Dietz, H. (2018). Sequence-programmable covalent bonding of designed DNA assemblies. Sci. Adv. *4*, eaau1157.

70. Conway, J.W., McLaughlin, C.K., Castor, K.J., and Sleiman, H. (2013). DNA nanostructure serum stability: greater than the sum of its parts. Chem. Commun. (Camb.) *49*, 1172–1174.

71. Perrault, S.D., and Shih, W.M. (2014). Virus-inspired membrane encapsulation of DNA nanostructures to achieve in vivo stability. ACS Nano *8*, 5132–5140.

72. Kim, Y., and Yin, P. (2020). Enhancing biocompatible stability of DNA nanostructures using dendritic oligonucleotides and brick motifs. Angew. Chem. Int. Ed. Engl. *59*, 700–703.

73. Wang, S.T., Gray, M.A., Xuan, S., Lin, Y., Byrnes, J., Nguyen, A.I., Todorova, N., Stevens, M.M., Bertozzi, C.R., Zuckermann, R.N., and Gang, O. (2020). DNA origami protection and molecular interfacing through engineered sequence-defined peptoids. Proc. Natl. Acad. Sci. USA *117*, 6339–6348.

74. Estrich, N.A., Hernandez-Garcia, A., de Vries, R., and LaBean, T.H. (2017). Engineered diblock polypeptides improve DNA and gold solubility during molecular assembly. ACS Nano *11*, 831–842.

75. Lacroix, A., Edwardson, T.G.W., Hancock, M.A., Dore, M.D., and Sleiman, H.F. (2017). Development of DNA nanostructures for high-affinity binding to human serum albumin. J. Am. Chem. Soc. *139*, 7355–7362.

76. Auvinen, H., Zhang, H., Nonappa, Kopilow, A., Niemelä, E.H., Nummelin, S., Correia, A., Santos, H.A., Linko, V., and Kostiainen, M.A. (2017). Protein coating of DNA nanostructures for enhanced stability and immunocompatibility. Adv. Healthc. Mater. *6*, 1700692.

77. Agarwal, N.P., Matthies, M., Gür, F.N., Osada, K., and Schmidt, T.L. (2017). Block copolymer micellization as a protection strategy for DNA origami. Angew. Chem. Int. Ed. Engl. *56*, 5460–5464.

78. Ponnuswamy, N., Bastings, M.M.C., Nathwani, B., Ryu, J.H., Chou, L.Y.T., Vinther, M., Li, W.A., Anastassacos, F.M., Mooney, D.J., and Shih, W.M. (2017). Oligolysine-based coating protects DNA nanostructures from low-salt denaturation and nuclease degradation. Nat. Commun. *8*, 15654.

79. Anastassacos, F.M., Zhao, Z., Zeng, Y., and Shih, W.M. (2020). Glutaraldehyde cross-linking of oligolysines coating DNA origami greatly reduces susceptibility to nuclease degradation. J. Am. Chem. Soc. *142*, 3311–3315.

80. Kiviaho, J.K., Linko, V., Ora, A., Tiainen, T., Järvihaavisto, E., Mikkilä, J., Tenhu, H., Nonappa, and Kostiainen, M.A. (2016). Cationic polymers for DNA origami coating - examining their binding efficiency and tuning the enzymatic reaction rates. Nanoscale *8*, 11674–11680.

81. Ahmadi, Y., De Llano, E., and Barišić, I. (2018). (Poly)cation-induced protection of conventional and wireframe DNA origami nanostructures. Nanoscale *10*, 7494–7504.

82. Lv, Y., Hu, R., Zhu, G., Zhang, X., Mei, L., Liu, Q., Qiu, L., Wu, C., and Tan, W. (2015). Preparation and biomedical applications of programmable and multifunctional DNA nanoflowers. Nat. Protoc. *10*, 1508–1524.

83. Liang, L., Li, J., Li, Q., Huang, Q., Shi, J., Yan, H., and Fan, C. (2014). Single-particle tracking and modulation of cell entry pathways of a tetrahedral DNA nanostructure in live cells. Angew Chem Int Ed Engl *53*, 7745–7750.

84. Wang, P., Rahman, M.A., Zhao, Z., Weiss, K., Zhang, C., Chen, Z., Hurwitz, S.J., Chen, Z.G., Shin, D.M., and Ke, Y. (2018). Visualization of the cellular uptake and trafficking of DNA origami nanostructures in cancer cells. J. Am. Chem. Soc. *140*, 2478–2484.

85. Chen, G., Liu, D., He, C., Gannett, T.R., Lin, W., and Weizmann, Y. (2015). Enzymatic synthesis of periodic DNA nanoribbons for intracellular pH sensing and gene silencing. J. Am. Chem. Soc. *137*, 3844–3851.

86. Zhou, J., Sun, L., Wang, L., Liu, Y., Li, J., Li, J., Li, J., and Yang, H. (2019). Self-assembled and size-controllable oligonucleotide nanospheres for effective antisense gene delivery through an endocytosis-independent pathway. Angew Chem Int Ed Engl *58*, 5236–5240.

87. Lee, D.S., Qian, H., Tay, C.Y., and Leong, D.T. (2016). Cellular processing and destinies of artificial DNA nanostructures. Chem. Soc. Rev. *45*, 4199–4225.

88. Walsh, A.S., Yin, H., Erben, C.M., Wood, M.J., and Turberfield, A.J. (2011). DNA cage delivery to mammalian cells. ACS Nano *5*, 5427–5432.

89. Ding, H., Li, J., Chen, N., Hu, X., Yang, X., Guo, L., Li, Q., Zuo, X., Wang, L., Ma, Y., and Fan, C.

(2018). DNA nanostructure-programmed like-charge attraction at the cell-membrane interface. ACS Cent. Sci. *4*, 1344–1351.

90. Bastings, M.M.C., Anastassacos, F.M., Ponnuswamy, N., Leifer, F.G., Cuneo, G., Lin, C., Ingber, D.E., Ryu, J.H., and Shih, W.M. (2018). Modulation of the cellular uptake of DNA origami through control over mass and shape. Nano Lett *18*, 3557–3564.

91. Fu, M., Dai, L., Jiang, Q., Tang, Y., Zhang, X., Ding, B., and Li, J. (2016). Observation of intracellular interactions between DNA origami and lysosomes by the fluorescence localization method. Chem. Commun. (Camb.) *52*, 9240–9242.

92. Lacroix, A., Vengut-Climent, E., de Rochambeau, D., and Sleiman, H.F. (2019). Uptake and fate of fluorescently labeled DNA nanostructures in cellular environments: a cautionary tale. ACS Cent. Sci. *5*, 882–891.

93. Jiang, D., Ge, Z., Im, H.J., England, C.G., Ni, D., Hou, J., Zhang, L., Kutyreff, C.J., Yan, Y., Liu, Y., et al. (2018). DNA origami nanostructures can exhibit preferential renal uptake and alleviate acute kidney injury. Nat. Biomed. Eng. *2*, 865–877.

94. Jiang, D., Im, H.J., Boleyn, M.E., England, C.G., Ni, D., Kang, L., Engle, J.W., Huang, P., Lan, X., and Cai, W. (2019). Efficient renal clearance of DNA tetrahedron nanoparticles enables quantitative evaluation of kidney function. Nano Res *12*, 637–642.

95. Wiraja, C., Zhu, Y., Lio, D.C.S., Yeo, D.C., Xie, M., Fang, W., Li, Q., Zheng, M., Van Steensel, M., Wang, L., et al. (2019). Framework nucleic acids as programmable carrier for transdermal drug delivery. Nat. Commun. *10*, 1147.

96. Ko, S., Liu, H., Chen, Y., and Mao, C. (2008). DNA nanotubes as combinatorial vehicles for cellular delivery. Biomacromolecules *9*, 3039–3043.

97. Mikkilä, J., Eskelinen, A.P., Niemelä, E.H., Linko, V., Frilander, M.J., Törmä, P., and Kostiainen, M.A. (2014). Virus-encapsulated DNA origami nanostructures for cellular delivery. Nano Lett *14*, 2196–2200.

98. Sefah, K., Shangguan, D., Xiong, X., O'Donoghue, M.B., and Tan, W. (2010). Development of DNA aptamers using CELL-SELEX. Nat. Protoc. *5*, 1169–1185.

99. Modi, S., Nizak, C., Surana, S., Halder, S., and Krishnan, Y. (2013). Two DNA nanomachines map pH changes along intersecting endocytic pathways inside the same cell. Nat. Nanotechnol. *8*, 459–467.

100. Lin, C., Wang, X., Liu, Y., Seeman, N.C., and Yan, H. (2007). Rolling circle enzymatic replication of a complex multi-crossover DNA nanostructure. J. Am. Chem. Soc. *129*, 14475–14481.

101. Li, Z., Wei, B., Nangreave, J., Lin, C., Liu, Y., Mi, Y., and Yan, H. (2009). A replicable tetrahedral nanostructure self-assembled from a single DNA strand. J. Am. Chem. Soc. *131*, 13093–13098.

102. Ducani, C., Kaul, C., Moche, M., Shih, W.M., and Högberg, B. (2013). Enzymatic production of 'monoclonal stoichiometric' single-stranded DNA oligonucleotides. Nat. Methods *10*, 647–652.

103. Kick, B., Praetorius, F., Dietz, H., and Weuster-Botz, D. (2015). Efficient production of single-stranded phage DNA as scaffolds for DNA origami. Nano Lett *15*, 4672–4676.

104. Minev, D., Guerra, R., Kishi, J.Y., Smith, C., Krieg, E., Said, K., Hornick, A., Sasaki, H.M., Filsinger, G., Beliveau, B.J., et al. (2019). Rapid in vitro production of single-stranded DNA. Nucleic Acids Res *47*, 11956–11962.

105. Marchi, A.N., Saaem, I., Vogen, B.N., Brown, S., and LaBean, T.H. (2014). Toward larger DNA origami. Nano Lett *14*, 5740–5747.

106. Schmidt, T.L., Beliveau, B.J., Uca, Y.O., Theilmann, M., Da Cruz, F., Wu, C.T., and Shih, W.M. (2015). Scalable amplification of strand subsets from chip-synthesized oligonucleotide libraries. Nat. Commun. *6*, 8634.

107. Praetorius, F., Kick, B., Behler, K.L., Honemann, M.N., Weuster-Botz, D., and Dietz, H. (2017). Biotechnological mass production of DNA origami. Nature *552*, 84–87.

108. Halley, P.D., Patton, R.A., Chowdhury, A., Byrd, J.C., and Castro, C.E. (2019). Low-cost, simple, and scalable self-assembly of DNA origami nanostructures. Nano Res *12*, 1207–1215.

109. Krieg, E., and Shih, W.M. (2018). Selective nascent polymer catch-and-release enables scalable isolation of multi-kilobase single-stranded DNA. Angew Chem Int Ed Engl *57*, 714–718.

110. Lin, C., Perrault, S.D., Kwak, M., Graf, F., and Shih, W.M. (2013). Purification of DNA-origami nanostructures by rate-zonal centrifugation. Nucleic Acids Res *41*, e40.

111. Stahl, E., Martin, T.G., Praetorius, F., and Dietz, H. (2014). Facile and scalable preparation of pure and dense DNA origami solutions. Angew Chem Int Ed Engl *53*, 12735–12740.

112. Kim, K.R., Kim, D.R., Lee, T., Yhee, J.Y., Kim, B.S., Kwon, I.C., and Ahn, D.R. (2013). Drug delivery by a self-assembled DNA tetrahedron for overcoming drug resistance in breast cancer cells. Chem. Commun (Camb.) *49*, 2010–2012.

113. Zhao, Y.X., Shaw, A., Zeng, X., Benson, E., Nyström, A.M., and Högberg, B. (2012). DNA origami delivery system for cancer therapy with tunable release properties. ACS Nano *6*, 8684–8691.

114. Jiang, Q., Song, C., Nangreave, J., Liu, X., Lin, L., Qiu, D., Wang, Z.-G., Zou, G., Liang, X., Yan, H., and Ding, B. (2012). DNA origami as a carrier for circumvention of drug resistance. J. Am. Chem. Soc. *134*, 13396–13403.

115. Halley, P.D., Lucas, C.R., McWilliams, E.M., Webber, M.J., Patton, R.A., Kural, C., Lucas, D.M., Byrd, J.C., and Castro, C.E. (2016). Daunorubicin-loaded DNA origami nanostructures circumvent drug-resistance mechanisms in a leukemia model. Small *12*, 308–320.

116. Zhou, F., Wang, P., Peng, Y., Zhang, P., Huang, Q., Sun, W., He, N., Fu, T., Zhao, Z., Fang, X., and Tan, W. (2019). Molecular engineering-based aptamer-drug conjugates with accurate tunability of drug ratios for drug combination targeted cancer therapy. Angew Chem Int Ed Engl *58*, 11661–11665.

117. Zhu, G., Hu, R., Zhao, Z., Chen, Z., Zhang, X., and Tan, W. (2013). Noncanonical self-assembly of multifunctional DNA nanoflowers for biomedical applications. J. Am. Chem. Soc. *135*, 16438–16445.

118. Hu, R., Zhang, X., Zhao, Z., Zhu, G., Chen, T., Fu, T., and Tan, W. (2014). DNA nanoflowers for multiplexed cellular imaging and traceable targeted drug delivery. Angew Chem Int Ed Engl *53*, 5821–5826.

119. Chang, M., Yang, C.S., and Huang, D.M. (2011). Aptamer-conjugated DNA icosahedral nanoparticles as a carrier of doxorubicin for cancer therapy. ACS Nano *5*, 6156–6163.

120. Yan, J., Hu, C., Wang, P., Zhao, B., Ouyang, X., Zhou, J., Liu, R., He, D., Fan, C., and Song, S. (2015). Growth and origami folding of DNA on nanoparticles for high-efficiency molecular transport in cellular imaging and drug delivery. Angew Chem Int Ed Engl *54*, 2431–2435.

121. Wu, T., Liu, J., Liu, M., Liu, S., Zhao, S., Tian, R., Wei, D., Liu, Y., Zhao, Y., Xiao, H., and Ding, B. (2019). A nanobody-conjugated DNA nanoplatform for targeted platinum-drug delivery. Angew Chem Int Ed Engl *58*, 14224–14228.

122. Sun, W., Jiang, T., Lu, Y., Reiff, M., Mo, R., and Gu, Z. (2014). Cocoon-like self-degradable DNA nanoclew for anticancer drug delivery. J. Am. Chem. Soc. *136*, 14722–14725.

123. Mo, R., Jiang, T., DiSanto, R., Tai, W., and Gu, Z. (2014). ATP-triggered anticancer drug delivery. Nat. Commun. *5*, 3364.

124. Zhang, L., Abdullah, R., Hu, X., Bai, H., Fan, H., He, L., Liang, H., Zou, J., Liu, Y., Sun, Y., et al. (2019). Engineering of bioinspired, size-controllable, self-degradable cancer-targeting DNA nanoflowers via the incorporation of an artificial sandwich base. J. Am. Chem. Soc. *141*, 4282–4290.

125. Wang, J., Wang, H., Wang, H., He, S., Li, R., Deng, Z., Liu, X., and Wang, F. (2019). Nonviolent self-catabolic DNAzyme nanosponges for smart anticancer drug delivery. ACS Nano *13*, 5852–5863.

126. Zhang, Q., Jiang, Q., Li, N., Dai, L., Liu, Q., Song, L., Wang, J., Li, Y., Tian, J., Ding, B., and Du, Y. (2014). DNA origami as an in vivo drug delivery vehicle for cancer therapy. ACS Nano *8*, 6633–6643.

127. Zhu, G., Zheng, J., Song, E., Donovan, M., Zhang, K., Liu, C., and Tan, W. (2013). Self-assembled, aptamer-tethered DNA nanotrains for targeted transport of molecular drugs in cancer theranostics. Proc. Natl. Acad. Sci. USA *110*, 7998–8003.

128. Mou, Q., Ma, Y., Pan, G., Xue, B., Yan, D., Zhang, C., and Zhu, X. (2017). DNA Trojan horses: self-assembled floxuridine-containing DNA polyhedra for cancer therapy. Angew Chem Int Ed Engl *56*, 12528–12532.

129. Zhang, J., Guo, Y., Ding, F., Pan, G., Zhu, X., and Zhang, C. (2019). A camptothecin-grafted DNA tetrahedron as a precise nanomedicine to inhibit tumor growth. Angew Chem Int Ed Engl *58*, 13794–13798.

130. Ma, Y., Wang, Z., Ma, Y., Han, Z., Zhang, M., Chen, H., and Gu, Y. (2018). A telomerase-responsive DNA icosahedron for precise delivery of platinum nanodrugs to cisplatin-resistant cancer. Angew Chem Int Ed Engl *57*, 5389–5393.

131. Keum, J.W., Ahn, J.H., and Bermudez, H. (2011). Design, assembly, and activity of antisense DNA nanostructures. Small *7*, 3529–3535.

132. Roh, Y.H., Lee, J.B., Shopsowitz, K.E., Dreaden, E.C., Morton, S.W., Poon, Z., Hong, J., Yamin, I., Bonner, D.K., and Hammond, P.T. (2014). Layer-by-layer assembled antisense DNA microsponge particles for efficient delivery of cancer therapeutics. ACS Nano *8*, 9767–9780.

133. Li, J., Zheng, C., Cansiz, S., Wu, C., Xu, J., Cui, C., Liu, Y., Hou, W., Wang, Y., Zhang, L., et al. (2015). Self-assembly of DNA nanohydrogels with controllable size and stimuli-responsive property for targeted gene regulation therapy. J. Am. Chem. Soc. *137*, 1412–1415.

134. Lee, H., Lytton-Jean, A.K., Chen, Y., Love, K.T., Park, A.I., Karagiannis, E.D., Sehgal, A., Querbes, W., Zurenko, C.S., Jayaraman, M., et al. (2012). Molecularly self-assembled nucleic acid nanoparticles for targeted in vivo siRNA delivery. Nat. Nanotechnol. *7*, 389–393.

135. Rahman, M.A., Wang, P., Zhao, Z., Wang, D., Nannapaneni, S., Zhang, C., Chen, Z., Griffith, C.C., Hurwitz, S.J., Chen, Z.G., et al. (2017). Systemic delivery of Bcl2-targeting siRNA by DNA nanoparticles suppresses cancer cell growth. Angew. Chem. Int. Ed. Engl. *56*, 16023–16027.

136. Sun, W., Ji, W., Hall, J.M., Hu, Q., Wang, C., Beisel, C.L., and Gu, Z. (2015). Self-assembled DNA nanoclews for the efficient delivery of CRISPR-Cas9 for genome editing. Angew Chem Int Ed Engl *54*, 12029–12033.

137. Setten, R.L., Rossi, J.J., and Han, S.P. (2019). The current state and future directions of RNAi-based therapeutics. Nat. Rev. Drug Discov. *18*, 421–446.

138. Shu, Z., Tanaka, I., Ota, A., Fushihara, D., Abe, N., Kawaguchi, S., Nakamoto, K., Tomoike, F., Tada, S., Ito, Y., et al. (2019). Disulfide-unit conjugation enables ultrafast cytosolic internalization of antisense DNA and siRNA. Angew Chem Int Ed Engl *58*, 6611–6615.

139. Lee, J.B., Hong, J., Bonner, D.K., Poon, Z., and Hammond, P.T. (2012). Self-assembled RNA interference microsponges for efficient siRNA delivery. Nat. Mater. *11*, 316–322.

140. Ding, F., Mou, Q., Ma, Y., Pan, G., Guo, Y., Tong, G., Choi, C.H.J., Zhu, X., and Zhang, C. (2018). A crosslinked nucleic acid nanogel for effective siRNA delivery and antitumor therapy. Angew Chem Int Ed Engl *57*, 3064–3068.

141. Ferreira, C.S., Cheung, M.C., Missailidis, S., Bisland, S., and Gariépy, J. (2009). Phototoxic aptamers selectively enter and kill epithelial cancer cells. Nucleic Acids Res *37*, 866–876.

142. Shieh, Y.A., Yang, S.J., Wei, M.F., and Shieh, M.J. (2010). Aptamer-based tumor-targeted drug delivery for photodynamic therapy. ACS Nano *4*, 1433–1442.

143. Han, D., Zhu, G., Wu, C., Zhu, Z., Chen, T., Zhang, X., and Tan, W. (2013). Engineering a cell-surface aptamer circuit for targeted and amplified photodynamic cancer therapy. ACS Nano *7*, 2312–2319.

144. You, M., Peng, L., Shao, N., Zhang, L., Qiu, L., Cui, C., and Tan, W. (2014). DNA "nano-claw": logic-based autonomous cancer targeting and therapy. J. Am. Chem. Soc. *136*, 1256–1259.

145. You, M., Zhu, G., Chen, T., Donovan, M.J., and Tan, W. (2015). Programmable and multiparameter DNA-based logic platform for cancer recognition and targeted therapy. J. Am. Chem. Soc. *137*, 667–674.

146. Zhuang, X., Ma, X., Xue, X., Jiang, Q., Song, L., Dai, L., Zhang, C., Jin, S., Yang, K., Ding, B., Wang, P.C., and Liang, X.-J. (2016). A photosensitizer-loaded DNA origami nanosystem for photodynamic therapy. ACS Nano *10*, 3486–3495.

147. Jiang, Q., Shi, Y., Zhang, Q., Li, N., Zhan, P., Song, L., Dai, L., Tian, J., Du, Y., Cheng, Z., and Ding, B. (2015). A self-assembled DNA origami-gold nanorod complex for cancer theranostics. Small *11*, 5134–5141.

148. Du, Y., Jiang, Q., Beziere, N., Song, L., Zhang, Q., Peng, D., Chi, C., Yang, X., Guo, H., Diot, G., et al. (2016). DNA-nanostructure-gold-nanorod hybrids for enhanced in vivo optoacoustic imaging and photothermal therapy. Adv. Mater. *28*, 10000–10007.

149. Song, L., Jiang, Q., Liu, J., Li, N., Liu, Q., Dai, L., Gao, Y., Liu, W., Liu, D., and Ding, B. (2017). DNA origami/gold nanorod hybrid nanostructures for the circumvention of drug resistance. Nanoscale *9*, 7750–7754.

150. Pan, M., Jiang, Q., Sun, J., Xu, Z., Zhou, Y., Zhang, L., and Liu, X. (2020). Programming DNA nanoassembly for enhanced photodynamic therapy. Angew Chem Int Ed Engl *59*, 1897–1905.

151. Krieg, A.M. (2006). Therapeutic potential of toll-like receptor 9 activation. Nat. Rev. Drug Discov. *5*, 471–484.

152. Nishikawa, M., Matono, M., Rattanakiat, S., Matsuoka, N., and Takakura, Y. (2008). Enhanced immunostimulatory activity of oligodeoxynucleotides by y-shape formation. Immunology *124*, 247–255.

153. Rattanakiat, S., Nishikawa, M., Funabashi, H., Luo, D., and Takakura, Y. (2009). The assembly of a short linear natural cytosine-phosphate-guanine DNA into dendritic structures and its effect on immunostimulatory activity. Biomaterials *30*, 5701–5706.

154. Nishikawa, M., Mizuno, Y., Mohri, K., Matsuoka, N., Rattanakiat, S., Takahashi, Y., Funabashi, H., Luo, D., and Takakura, Y. (2011). Biodegradable CpG DNA hydrogels for sustained delivery of doxorubicin and immunostimulatory signals in tumor-bearing mice. Biomaterials *32*, 488–494.

155. Li, J., Pei, H., Zhu, B., Liang, L., Wei, M., He, Y., Chen, N., Li, D., Huang, Q., and Fan, C. (2011). Self-assembled multivalent DNA nanostructures for noninvasive intracellular delivery of immunostimulatory CpG oligonucleotides. ACS Nano *5*, 8783–8789.

156. Schüller, V.J., Heidegger, S., Sandholzer, N., Nickels, P.C., Suhartha, N.A., Endres, S., Bourquin, C., and Liedl, T. (2011). Cellular immunostimulation by CpG-sequence-coated DNA origami structures. ACS Nano *5*, 9696–9702.

157. Liu, X., Xu, Y., Yu, T., Clifford, C., Liu, Y., Yan, H., and Chang, Y. (2012). A DNA nanostructure platform for directed assembly of synthetic vaccines. Nano Lett *12*, 4254–4259.

158. Douglas, S.M., Bachelet, I., and Church, G.M. (2012). A logic-gated nanorobot for targeted transport of molecular payloads. Science *335*, 831–834.

159. Li, S., Jiang, Q., Liu, S., Zhang, Y., Tian, Y., Song, C., Wang, J., Zou, Y., Anderson, G.J., Han, J.-Y., et al. (2018). A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger in vivo. Nat. Biotechnol. *36*, 258–264.

160. Wang, C., Sun, W., Wright, G., Wang, A.Z., and Gu, Z. (2016). Inflammation-triggered cancer immunotherapy by programmed delivery of CpG and anti-PD1 antibody. Adv. Mater. *28*, 8912–8920.

161. Zhang, D., Zheng, Y., Lin, Z., Liu, X., Li, J., Yang, H., and Tan, W. (2020). Equipping natural killer cells with specific targeting and checkpoint blocking aptamers for enhanced adoptive immunotherapy in solid tumors. Angew Chem Int Ed Engl *59*, 12022–12028.

162. Yang, Y., Sun, X., Xu, J., Cui, C., Safari Yazd, H., Pan, X., Zhu, Y., Chen, X., Li, X., Li, J., and Tan, W. (2020). Circular bispecific aptamer-mediated artificial intercellular recognition for targeted T cell immunotherapy. ACS Nano *14*, 9562–9571.

163. Liu, J., Song, L., Liu, S., Jiang, Q., Liu, Q., Li, N., Wang, Z.G., and Ding, B. (2018). A DNA-based nanocarrier for efficient gene delivery and combined cancer therapy. Nano Lett *18*, 3328–3334.

164. Wu, C., Han, D., Chen, T., Peng, L., Zhu, G., You, M., Qiu, L., Sefah, K., Zhang, X., and Tan, W. (2013). Building a multifunctional aptamer-based DNA nanoassembly for targeted cancer therapy. J. Am. Chem. Soc. *135*, 18644–18650.

165. Liu, J., Song, L., Liu, S., Zhao, S., Jiang, Q., and Ding, B. (2018). A tailored DNA nanoplatform for synergistic RNAi-/chemotherapy of multidrug-resistant tumors. Angew Chem Int Ed Engl *57*, 15486–15490.

166. Blanco, E., Shen, H., and Ferrari, M. (2015). Principles of nanoparticle design for overcoming biological barriers to drug delivery. Nat. Biotechnol. *33*, 941–951.

167. Kamaly, N., He, J.C., Ausiello, D.A., and Farokhzad, O.C. (2016). Nanomedicines for renal disease: current status and future applications. Nat. Rev. Nephrol. *12*, 738–753.

168. Denby, L., and Baker, A.H. (2016). Targeting non-coding RNA for the therapy of renal disease. Curr. Opin. Pharmacol. *27*, 70–77.

169. Vaidya, V.S., Ozer, J.S., Dieterle, F., Collings, F.B., Ramirez, V., Troth, S., Muniappa, N., Thudium, D., Gerhold, D., Holder, D.J., et al. (2010). Kidney injury molecule-1 outperforms traditional biomarkers of kidney injury in preclinical biomarker qualification studies. Nat. Biotechnol. *28*, 478–485.

170. Dieterle, F., Perentes, E., Cordier, A., Roth, D.R., Verdes, P., Grenet, O., Pantano, S., Moulin, P., Wahl, D., Mahl, A., et al. (2010). Urinary clusterin, cystatin C, beta2-microglobulin and total protein as markers to detect drug-induced kidney injury. Nat. Biotechnol. *28*, 463–469.

171. Meng, X.-M., Nikolic-Paterson, D.J., and Lan, H.Y. (2016). TGF-β: the master regulator of fibrosis. Nat. Rev. Nephrol. *12*, 325–338.

172. Ijäs, H., Shen, B., Heuer-Jungemann, A., Keller, A., Kostiainen, M.A., Liedl, T., Ihalainen, J.A., and Linko, V. (2020). Unraveling the Interaction between Doxorubicin and DNA Origami Nanostructures for Customizable Chemotherapeutic Drug Release. bioRxiv. https://doi.org/10.1101/2020.05.13.088054.

173. Surana, S., Shenoy, A.R., and Krishnan, Y. (2015). Designing DNA nanodevices for compatibility with the immune system of higher organisms. Nat. Nanotechnol. *10*, 741–747.

174. Kwon, P.S., Ren, S., Kwon, S.J., Kizer, M.E., Kuo, L., Xie, M., Zhu, D., Zhou, F., Zhang, F., Kim, D., et al. (2020). Designer DNA architecture offers precise and multivalent spatial pattern-recognition for viral sensing and inhibition. Nat. Chem. *12*, 26–35.

175. Veneziano, R., Moyer, T.J., Stone, M.B., Wamhoff, E.C., Read, B.J., Mukherjee, S., Shepherd, T.R., Das, J., Schief, W.R., Irvine, D.J., and Bathe, M. (2020). Role of nanoscale antigen organization on B-cell activation probed using DNA origami. Nat. Nanotechnol. *15*, 716–723.