Review

Nano Biomed Eng

DNA Nanocarriers for Delivery of sgRNA/Cas9 Ribonucleoprotein

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Abstract

DNA has been widely employed as a building block for the construction of sophisticated nanostructures with pre-designed sizes and shapes by complementary base pairing. With outstanding programmability, addressability, and biocompatibility, DNA nanostructures have been further developed as nanocarriers for drug delivery in biomedical researches. Noticeably, DNA nanocarriers can be rationally designed for loading and delivering nucleic acid drugs based on their inherent homology. Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas9) ribonucleoprotein-based gene editing system has also been efficiently delivered by DNA nanocarriers. In this review, we will summarize the recent progress in the design of versatile DNA nanocarriers, such as rolling circle amplification (RCA)-based DNA nanostructure, branched DNA, and DNA origami, for delivery of single-guide RNA (sgRNA)/Cas9 ribonucleoprotein. Furthermore, the challenges and future opportunities of DNA nanotechnology in the delivery of gene editing system will be discussed.

Keywords: DNA nanocarrier; drug delivery; sgRNA/Cas9 ribonucleoprotein; gene editing; gene therapy

Introduction

DNA, as a carrier of genetic information, has played a significant role in various life processes. Four kinds of nucleotides (A, T, G, and C) are included in the DNA sequences. Based on the classic rule of complementary base pairing, two single-stranded DNAs can be assembled together to form a double-helix structure, termed DNA duplex [1]. The genetic information of double-stranded DNA can be transferred into the mRNA, and then translated into the down-stream target protein, known as the central dogma of life [2]. The strict rule of complementary base pairing ensures the precise transmission of genetic information. Fortunately, the rule of complementary base pairing can be further employed to precisely organize multiple single-stranded DNA sequences into pre-designed nanostructures with

excellent programmability.

With the rapid development of DNA nanotechnology, a variety of DNA nanostructures have been constructed by different assembly strategies. In the early 1980s, Seeman, the pioneer of DNA nanotechnology, initially proposed that DNA could be utilized as the building block for the assembly of nanostructures (Fig. 1(a)) [3]. Depending on this strategy, a plenty of two-dimensional DNA nanostructures were fabricated [4–12]. In addition, the three-dimensional DNA polyhedral nanostructures were precisely designed and assembled with different morphologies [13–15]. Meanwhile, rolling circle amplification (RCA) is another strategy to obtain various sophisticated DNA structures (Fig. 1(b)) [16–19]. Furthermore, chemically modified DNA has also been utilized as the functional building block. Especially, the chemically conjugated branched DNA structures played an essential role in the assembly of multifunctional DNA nanostructures (Fig. 1(c)) [20–28]. Besides, in 2006, Rothmund opened up a new window for the construction of a totally new kind of DNA nanostructure, named DNA origami (Fig. 1(d)) [6]. In this strategy, a long single-stranded circular DNA (scaffold) was folded by hundreds of pre-designed short DNA oligonucleotides (staple strands) into various sophisticated nanostructures in one-pot [29–35]. With structural programmability, spatial addressability, and excellent biocompatibility, DNA nanostructures mentioned above have been widely employed in various research fields, such as precise fabrication, information storage, bio-imaging, and drug delivery [36–56].

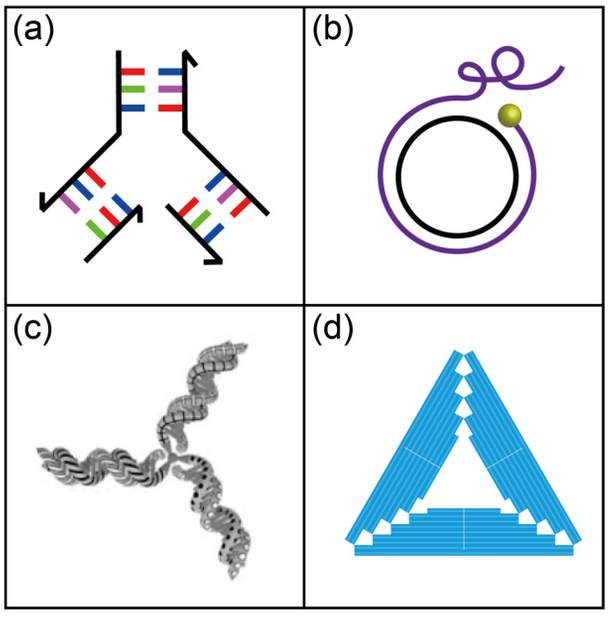

Fig. 1 Representative DNA nanostructures. **(a)** DNA junction. Reproduced with permission from Ref. [3] © 1982 Elsevier B.V. **(b)** RCA-based DNA structure. Reproduced with permission from Ref. [4] © 2014 Royal Society of Chemistry. **(c)** Branched DNA. Reproduced with permission from Ref. [5] © 2002 Nature Publishing Group. **(d)** DNA origami. Reproduced with permission from Ref. [6] © 2006 Nature Publishing Group.

CRISPR/Cas9 Gene Editing System

Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein (Cas9)-based gene editing system is an efficient molecular biological tool for the editing of target gene *in vitro* and *in vivo* [57–66]. The single-guide RNA (sgRNA, for sequence-specific recognition of target gene) and Cas9 protein (for the subsequent cleavage of the recognized gene) were designed and constructed to form sgRNA/Cas9 ribonucleoprotein for targeted gene editing (Fig. 2).

Till now, three delivery modes of the CRISPR/Cas9 gene editing system have been established, including the plasmid vector encoding sgRNA and Cas9 protein, the transcribed sgRNA mixed with Cas9 mRNA, and the sgRNA/Cas9 ribonucleoprotein. Considering the long-term protein expression of plasmid vector and the instability of the long mRNA, the sgRNA/Cas9 ribonucleoprotein is preferred without the need for additional transcription and translation in cells. To achieve the efficient gene editing based on sgRNA/Cas9 ribonucleoprotein, the construction of a reliable delivery system is the first key step. To address this issue, a variety of delivery vehicles have been rationally designed and constructed, such as liposomes, polymers, and inorganic nanoparticles [67–79]. The sgRNA/Cas9

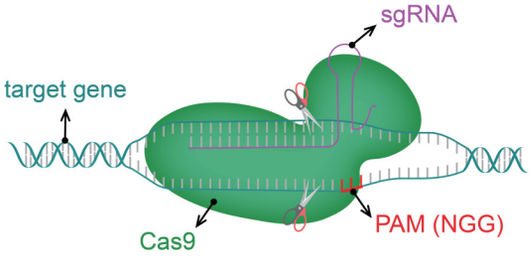

Fig. 2 Schematic illustration of the recognition and targeted gene editing by sgRNA/Cas9 ribonucleoprotein.

ribonucleoprotein can be loaded in these nanocarriers by electrostatic interaction, physical encapsulation, or chemical conjugation for efficient delivery. However, some of the exogenous components involved in these delivery systems, such as amphiphilic lipids, cationic polymers, and heavy metal ions, may induce systemic toxicity *in vivo*.

DNA Nanocarriers for sgRNA/Cas9 Ribonucleoprotein

Due to the inherent homology and excellent biocompatibility, DNA nanocarriers can be widely employed to load a series of nucleic acid drugs through complementary base pairing to achieve efficient gene therapy. The sgRNA involved in the sgRNA/Cas9 ribonucleoprotein can be exploited as the hybridization strand to be precisely organized in the pre-designed sites of DNA nanocarriers. Furthermore, the active targeting groups (such as small molecules, aptamers, and antibodies) and stimuli-responsive components (such as response to glutathione (GSH), pH, and nuclease) can be site-specifically included in the DNA nanocarriers for targeted delivery and controlled release of the loaded sgRNA/Cas9 ribonucleoprotein *in vitro* and *in vivo*. These multifunctional DNA structure-based nanocarriers have realized the efficient gene editing of different targeted genes.

RCA-based DNA nanocarriers

RCA is an efficient strategy to prepare the ultra-long single-stranded DNA with numerous tandem complementary copies of circular templates with the assistance of DNA polymerase. The RCA-based nanostructures have been widely employed in various biomedical applications [4, 80, 81]. These DNA aptamers, stimuli-responsive DNA sequences, and sgRNA complementary sequences can be introduced in the circular DNA template for efficient loading, targeted delivery and controlled release.

In 2015, Gu et al. designed a circular DNA template including a region for partially complementary base pairing with sgRNA for RCA to realize the controlled delivery of sgRNA/Cas9 ribonucleoprotein (Fig. 3) [82]. The tailored circular DNA template with palindromic sequences was first constructed for rolling circle amplification. After RCA, a yarn-like DNA nanoclew was efficiently synthesized. The sgRNA/Cas9 ribonucleoprotein can be easily loaded in the DNA nanoclew by partially complementary base pairing between the sgRNA and RCA-based DNA sequences. After loading of the sgRNA/Cas9 ribonucleoprotein, the DNA nanoclew was further coated with the cationic polymer polyethylenimine (PEI) for subsequent cellular uptake and endosomal escape. The escaped sgRNA/Cas9

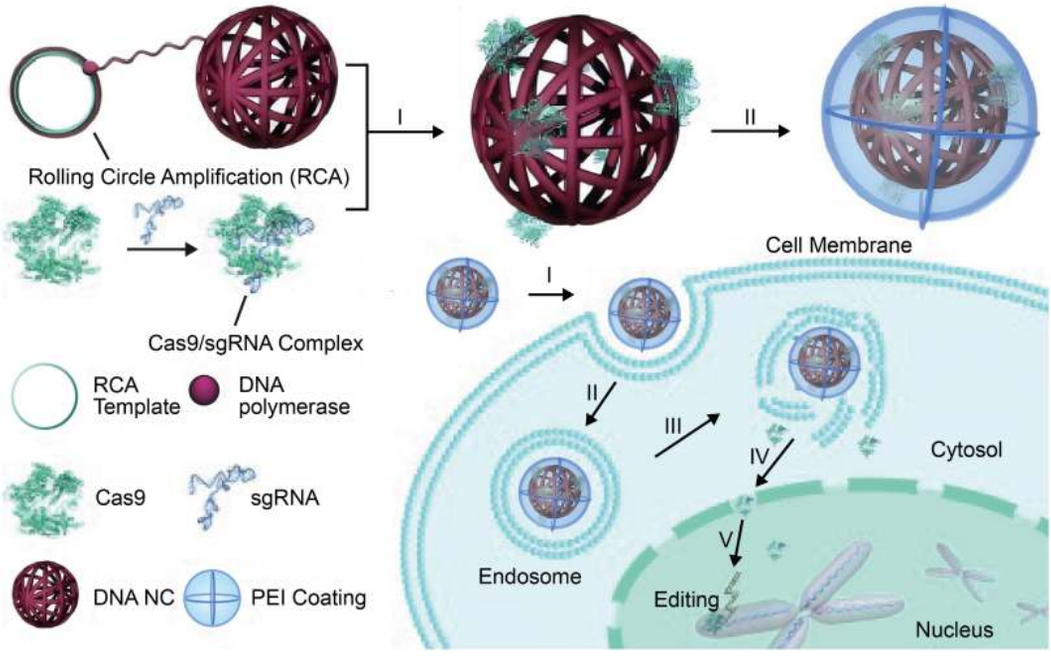

Fig. 3 RCA-based nanoclew for loading and delivery of sgRNA/Cas9 ribonucleoprotein. Reproduced with permission from Ref. [82] © 2015 Wiley-VCH.

ribonucleoprotein in the cytoplasm can be subsequently delivered into the cell nucleus with the guidance of nuclear localization signal peptide fused in Cas9 protein. The sgRNA/Cas9 ribonucleoprotein-loaded DNA nanoclew can achieve an efficient disruption of EGFP gene in the tumor cells with the stable expression of EGFP. This RCA-based gene editing system provided a promising strategy for loading and controlled delivery of sgRNA/Cas9 ribonucleoprotein *in vitro* and *in vivo*.

In 2020, Zhang et al. constructed a DNA nanoflower by RCA for targeted delivery and controlled release of sgRNA/Cas9 ribonucleoprotein (Fig. 4) [83]. A circular DNA template was first prepared for rolling circle amplification to obtain multiple replicates of DNA aptamers and miR-21 binding sequences in the DNA nanoflower. To realize the efficient loading and toehold-mediated release, an additional RNA sequence, which is 7 nt shorter than miR-21, was inserted into the stem-loop of sgRNA. The added RNA sequence didn't affect the cleavage activity of sgRNA/Cas9 ribonucleoprotein. Next, the rationally designed DNA nanoflower can recognize and load the newly prepared sgRNA/Cas9 ribonucleoprotein by RNA/DNA hybridization. Meanwhile, the multivalent MUC1 aptamers encoded on DNA nanoflower are responsible for specific cell targeting. The drug-loaded DNA nanoflower can go through the DNA aptamer-mediated endocytosis and Mg^{2+}-induced endosomal escape in the cytoplasm. After the targeted delivery and successful escape in tumor cells, the sgRNA/Cas9 ribonucleoprotein can be replaced by miR-21 through toehold-mediated sequence displacement. The stimuli-responsively released sgRNA/Cas9 ribonucleoprotein can be further transported into the nucleus with assistance from nuclear localization signal peptide fused in Cas9 for efficient gene editing *in vitro* and *in vivo*. This targeting group-modified and stimuli-responsive RCA-based gene editing system demonstrated an attractive solution for the targeted delivery and controlled release of sgRNA/Cas9 ribonucleoprotein to achieve an effective gene editing.

In 2022, Yang et al. reported a proton-activatable DNA-based nanocarrier to co-deliver sgRNA/Cas9 ribonucleoprotein and DNAzyme for combined gene therapy (Fig. 5) [84]. Multiple repeated recognition sequences of sgRNA, DNAzyme sequences, and Hha I enzyme cleavage sites were included in an ultra-long single-stranded DNA generated by rolling circle amplification. The sgRNA recognition sequences can specifically capture sgRNA/Cas9 ribonucleoprotein by RNA/DNA hybridization. The Mn^{2+} ions were included as the cofactor of DNAzyme and also played a role in condensing the drug-loaded DNA chains into nanoparticles. In the assembly process, the concentration of Mn^{2+} ions can be optimized to balance the activity of the Cas9 and DNAzyme. After loading and condensing, an acid-degradable polymer (poly-glycerol dimethacrylate)-coated Hha I enzyme

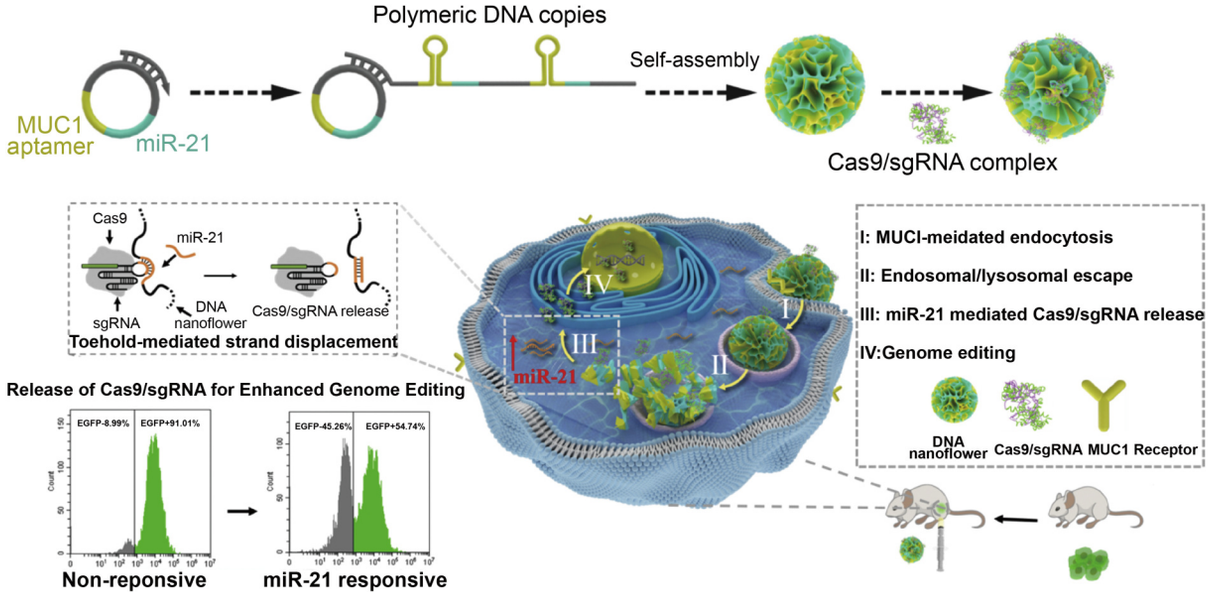

Fig. 4 RCA-based nanoflower for delivery of sgRNA/Cas9 ribonucleoprotein with a microRNA-responsive release behavior. Reproduced with permission from Ref. [83] © 2020 Elsevier B.V.

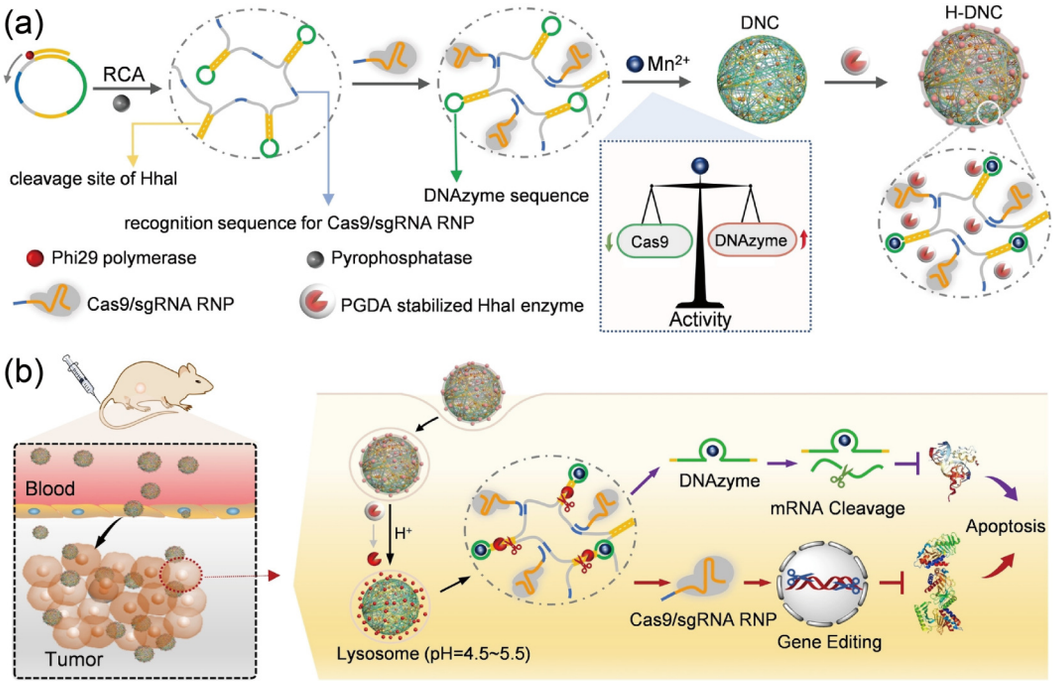

Fig. 5 (a) Construction of RCA-based nanocarrier for co-delivery of sgRNA/Cas9 ribonucleoprotein and DNAzyme. **(b)** The designed gene therapeutic process of this delivery system in tumor cells. Reproduced with permission from Ref. [84] © 2022 Wiley-VCH.

was assembled on the surface as the corona of the nanoparticle to construct the proton-activatable DNA nanocarrier. After incubation with tumor cells, the DNA nanocarrier can be easily internalized through lysosome mediated pathway. Under the acidic environment in lysosome, the outermost polymer was gradually degraded. Next, the exposed Hha I enzyme can recognize and cut off the cleavage sites in the functional DNA nanoparticles to release the sgRNA/Cas9 ribonucleoprotein and DNAzyme with the cofactor of Mn^{2+}. The released sgRNA/Cas9 ribonucleoprotein and DNAzyme can induce a remarkable gene editing and gene silencing effect *in vitro* and *in vivo*. This proton-activatable DNA nanocarrier of both sgRNA/Cas9 ribonucleoprotein and DNAzyme presented an inspiring approach for co-delivery of multiple gene therapeutic drugs to achieve the combined gene therapy.

Branched DNA-based nanocarriers

Terminal-modified DNA (containing amino, thiol, azide, or alkyne group) can conjugate with branched organic molecules to construct the branched DNA with indicated numbers of DNA arms for the subsequent assembly of crosslinked DNA materials. These covalently conjugated branched DNA nanostructures can be designed for the loading and delivery of nucleic acid drugs through complementary base pairing [28, 85, 86].

In 2019, Zhang et al. reported a non-cationic DNA-crosslinked nanogel for intracellular delivery of sgRNA/Cas9 ribonucleoprotein (Fig. 6) [87]. A DNA-grafted polycaprolactone brush (DNA-g-PCL) was first efficiently synthesized by the copper-free click reaction between azide-modified polycaprolactone and dibenzocyclooctyne-terminated DNA (DBCO-DNA). The DNA sequence of the obtained branched DNA-g-PCL was pre-designed to be complementary to the tail of sgRNA for the loading of sgRNA/Cas9 ribonucleoprotein. After loading, a DNA linker was included for further crosslinking to form a DNA nanogel by DNA hybridization. The loaded sgRNA/Cas9 ribonucleoprotein was fully embedded in the DNA nanogel to enhance the tolerance against nuclease digestion. After cellular uptake, the DNA nanogel can be gradually degraded by the nuclease, such as DNase I, for continuous release of the embedded sgRNA/Cas9 ribonucleoprotein. Based on the assembled compact architecture, the sgRNA/Cas9 ribonucleoprotein-loaded DNA nanogel presented an improved cellular uptake to achieve an efficient gene editing *in vitro* and successfully knocked down the EGFP expression in tumor cells. This DNA nanogel-based gene editing system provided a promising delivery tool for sgRNA/Cas9 ribonucleoprotein to

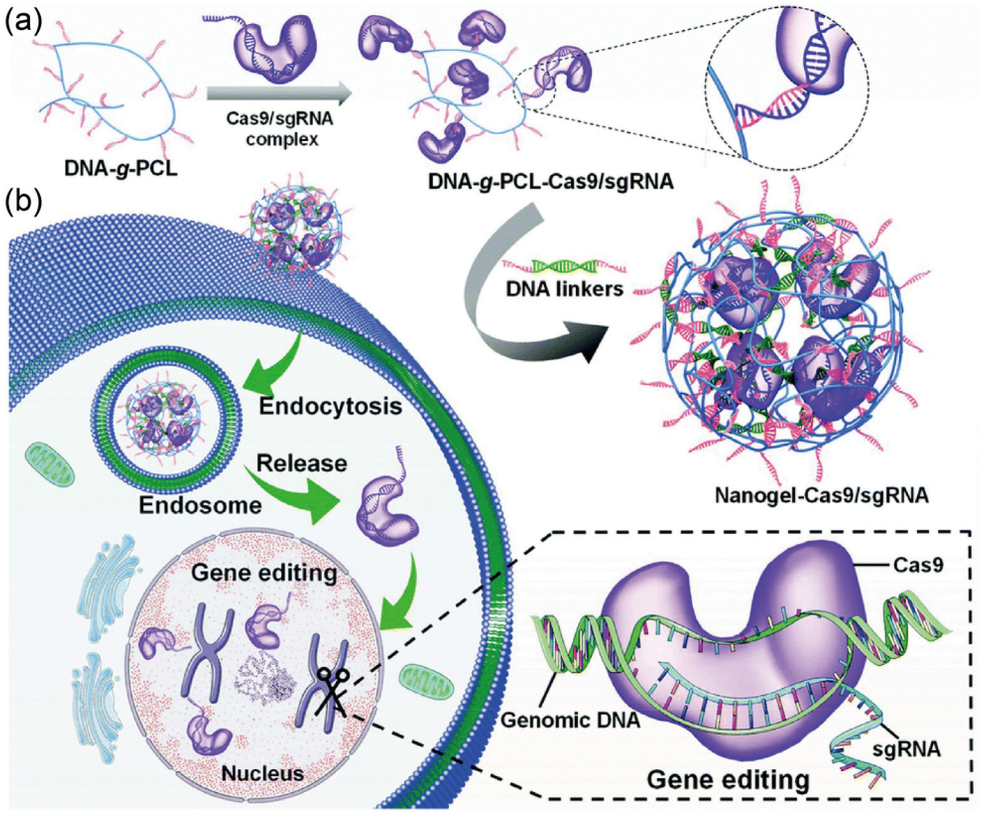

Fig. 6 (a) Construction of branched DNA-based nanogel for delivery of sgRNA/Cas9 ribonucleoprotein. **(b)** The designed gene editing process of this delivery system in cells. Reproduced with permission from Ref. [87] © 2019 Royal Society of Chemistry.

elicit an efficient and long-lasting gene editing effect *in vitro*.

Meanwhile, Ding et al. constructed a branched DNA-based nanoplatform for the co-delivery of gene editing system (sgRNA/Cas9 ribonucleoprotein, targeting genome in the nucleus) and gene silencing component (antisense, targeting mRNA in the cytoplasm) for synergistic tumor therapy *in vitro* and *in vivo* (Fig. 7) [88]. The azide-modified organic macromolecule ($7N_3$-β-Cyclodextrin) was first synthesized for chemical conjugation with the DBCO-DNA to form the branched DNA. Next, a DNA linker with two disulfide bonds and an antisense strand was pre-designed to be complementary to the 3' terminal extended sgRNA for co-assembly of branched DNA-based sgRNA/Cas9/antisense complex. Based on the host–guest interaction, the adamantane-modified DNA aptamer and endosomal escape peptide can be efficiently included. The aptamer-modified nanoplatform showed a much enhanced cellular uptake than the control groups. After GSH and RNase H digestion, the antisense and sgRNA/Cas9 ribonucleoprotein can be efficiently released to achieve the combined gene editing and gene silencing *in vivo*. This branched DNA-based nanoplatform demonstrated an attractive strategy for co-delivery of nucleic acid drugs for gene therapy.

In 2023, Yang et al. described a precipitation polymerization-based DNA nanoframework for controlled delivery of sgRNA/Cas9 ribonucleoprotein (Fig. 8) [89]. Acrylamide-modified DNA, disulfide bond-containing N,N-bis(acryloyl)cystamine (BACA), and N-isopropylacrylamide (NIPAM) were employed as the monomers for precipitation polymerization to construct the branched DNA-based DNA nanoframework. The partially complementary double-stranded DNA involved in the DNA nanoframework can function as an initiator for cascade hybridization chain reaction. Meanwhile, the hydrophilic acylamino and hydrophobic isopropyl included in this system are responsible for dynamic swelling and aggregation. So, the DNA nanoframework can swell below lower critical solution temperature. Under this condition, the sgRNA/Cas9 ribonucleoprotein can be efficiently loaded in the expanded DNA nanoframework by RNA/DNA hybridization. The high protein activity of Cas9 was also maintained during the loading process. Next, the introduced disulfide bond can be reduced by GSH in tumor cells for the subsequent disassembly of

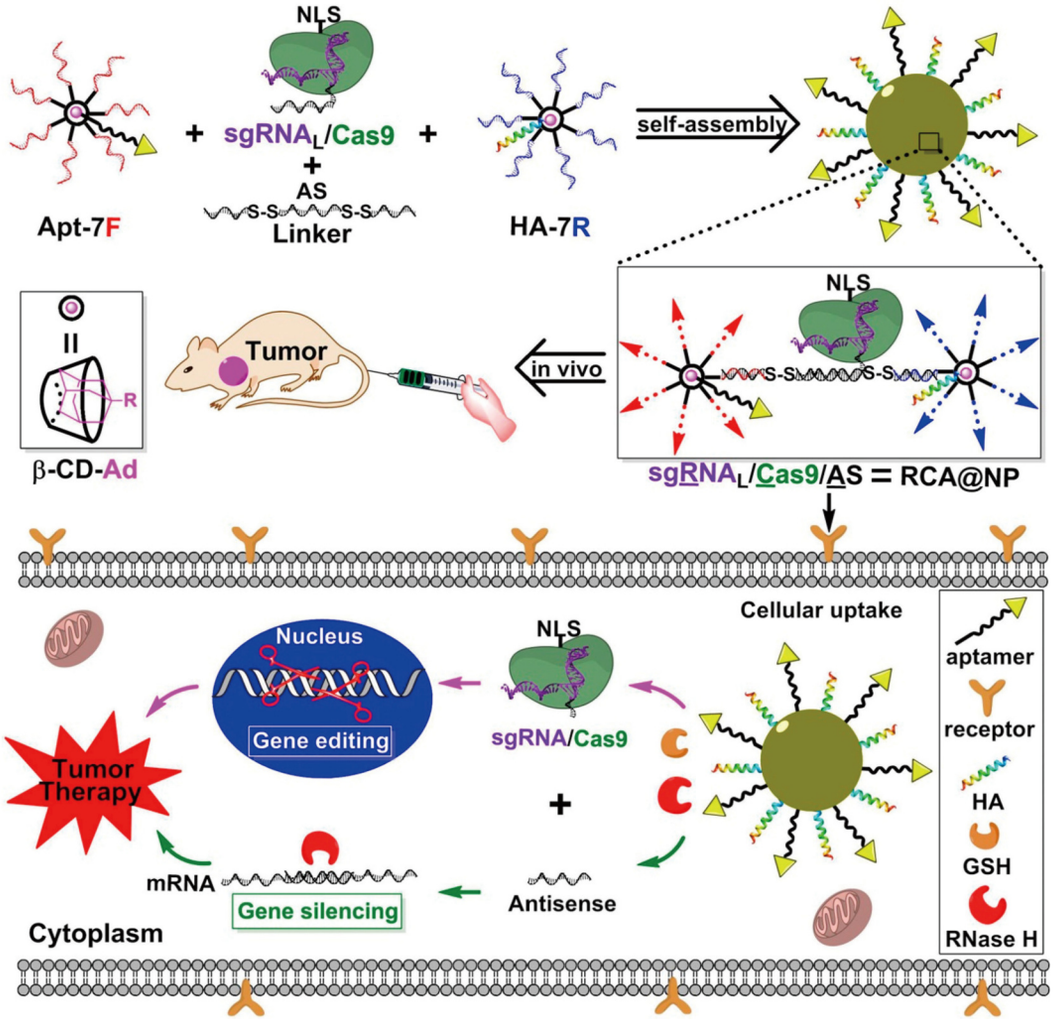

Fig. 7 A self-assembled platform based on branched DNA for co-delivery of sgRNA/Cas9 ribonucleoprotein and antisense to achieve synergistic tumor therapy *in vivo*. Reproduced with permission from Ref. [88] © 2019 American Chemical Society.

the DNA nanoframework. The exposed sgRNA/Cas9 ribonucleoprotein can be further released by the RNase H-based digestion of the RNA strand in the RNA/DNA hybrid duplex. After administration of the sgRNA/Cas9 ribonucleoprotein-loaded DNA nanoframework, a high gene editing efficiency was observed *in vitro* and *in vivo*. Noticeably, the remarkable biocompatibility and low side effects were also verified in normal cells and major organs *in vivo*. This chemically conjugated DNA nanoframework-based gene editing system presented a hopeful nanoplaform for precise gene editing *in vivo*.

DNA origami-based nanocarriers

With the excellent structural programmability and spatial addressability, DNA origami has been rationally designed for the precise organization of multiple cargoes [90–101]. The sgRNA/Cas9 ribonucleoprotein can be loaded and protected by the structurally well-defined DNA origami for drug delivery.

In 2023, Ding et al. reported a DNA origami-based gene editing system for gene therapy *in vivo* (Fig. 9) [102]. The protospacer-adjacent motif (PAM)-rich region (6× NGG) was precisely organized on the surface of DNA origami to recruit the sgRNA/Cas9 ribonucleoprotein by PAM-guided assembly. Meanwhile, the recruited sgRNA/Cas9 ribonucleoprotein can be further loaded by the hybridization between pre-designed DNA capture strands and the 3' terminal extended sgRNA. After that, the drug-loaded DNA origami can be rolled up by the locking strands with a disulfide bond. For targeted delivery and subsequent endosomal escape, the DNA aptamer (targeting a glycoprotein Mucin 1 overexpressed on the cell membrane of tumor cells) and influenza hemagglutinin peptide were site-specifically attached at the terminals of DNA

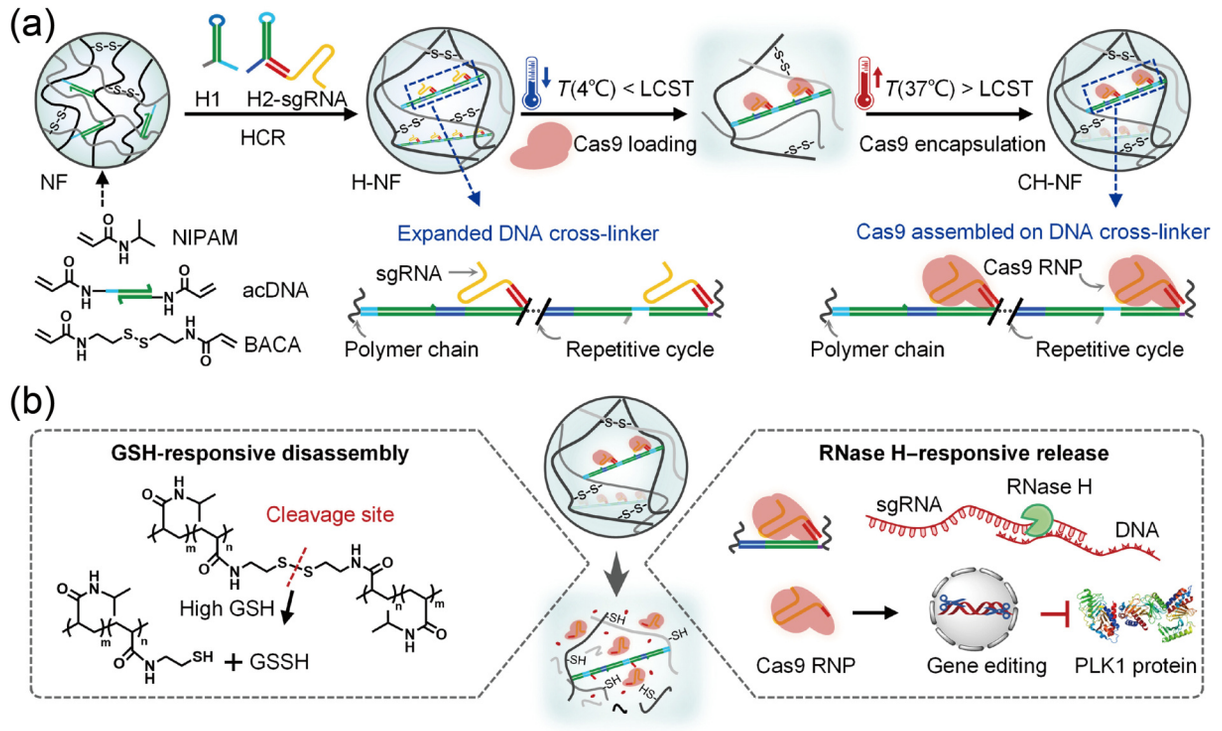

Fig. 8 (a) Cascade dynamic assembly/disassembly of branched DNA-based nanoframework for delivery of sgRNA/Cas9 ribonucleoprotein. **(b)** GSH-responsive disassembly and RNase H-responsive release of sgRNA/Cas9 ribonucleoprotein in tumor cells. Reproduced with permission from Ref. [89] © 2023 American Association for the Advancement of Science.

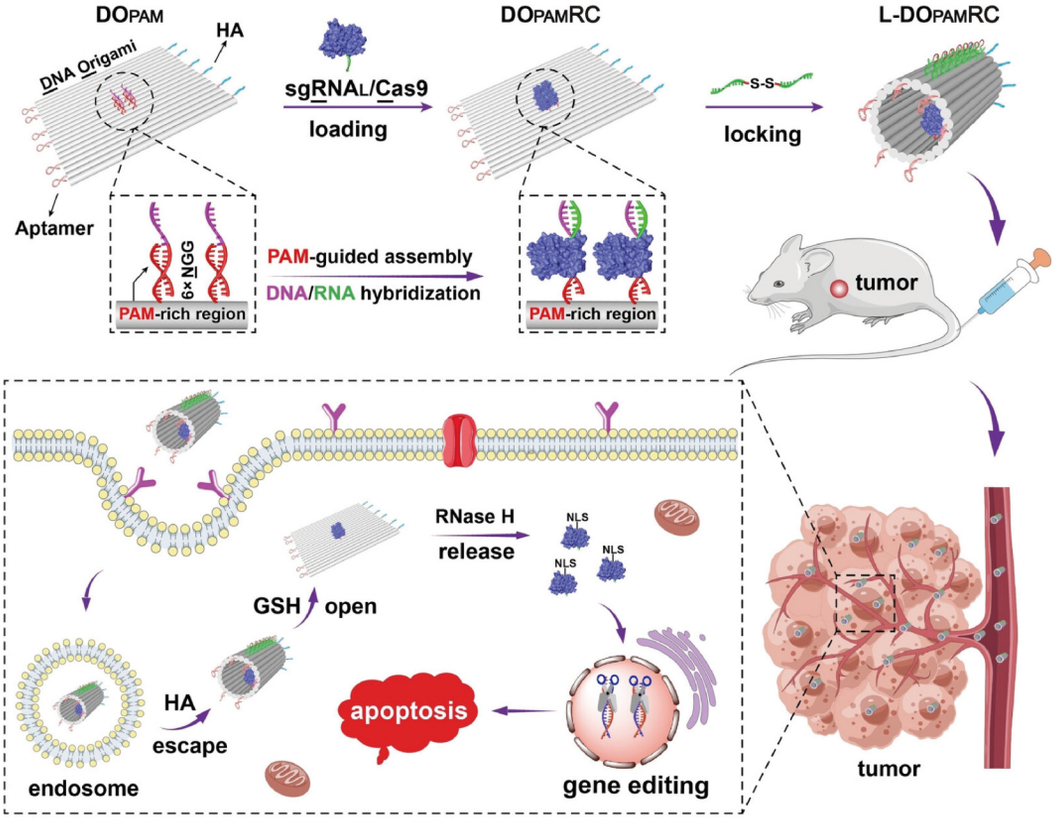

Fig. 9 DNA origami with PAM-rich region for loading and delivery of sgRNA/Cas9 ribonucleoprotein for gene therapy *in vivo*. Reproduced with permission from Ref. [102] © 2023 Wiley-VCH.

origami. After cellular uptake and endosomal escape, the locked structure can be opened by GSH reduction in the cytoplasm. Next, the RNase H is responsible for cleaving the RNA strand in the RNA/DNA hybrid duplex to release the sgRNA/Cas9 ribonucleoprotein. The released sgRNA/Cas9 ribonucleoprotein can enter the nucleus for targeted gene editing by the guidance of nuclear localization signal peptide attached in the Cas9 protein. After administration of this DNA origami-based gene editing system, a remarkable anti-tumor effect by disrupting the tumor-associated gene was observed *in vivo*. This DNA origami-based gene editing system provided a promising solution for targeted delivery and controlled release of sgRNA/Cas9 ribonucleoprotein to achieve efficient gene therapy *in vivo*.

In addition, Min et al. constructed a dual-chain-locked DNA origami nanocage for the delivery of sgRNA/Cas9 ribonucleoprotein (Fig. 10) [103]. The sgRNA/Cas9 ribonucleoprotein was first loaded in the inner cavity of DNA origami nanocage by RNA/DNA hybridization. After loading, the ATP and miRNA-21 responsive double-stranded DNAs were rationally designed to lock the nanocage. During transportation, the locked DNA origami-based nanocarrier can protect the loaded sgRNA/Cas9 ribonucleoprotein from deactivation and premature release. After internalization into tumor cells, the locking chains can be dismounted in response to molecular triggers (ATP and miRNA-21). The sgRNA/Cas9 ribonucleoprotein can be further released from the DNA origami nanocage through digesting the RNA strand of the RNA/DNA hybrid sequence by RNase H. Based on this stimuli-responsive design, whether and how much of the target gene can be edited by sgRNA/Cas9 ribonucleoprotein was solely determined by the level of biological molecular triggers in the pathophysiological environment of target sites. After administration of this DNA origami nanocage-based gene editing system targeting a tumor-related gene, a superior inhibition of the proliferation of tumor cells was achieved *in vitro* and *in vivo*. This stimuli-responsive DNA origami nanocage-based gene editing system demonstrated an attractive platform for precise gene editing in response to disease-associated biomarkers.

As shown in Table 1, these representative DNA

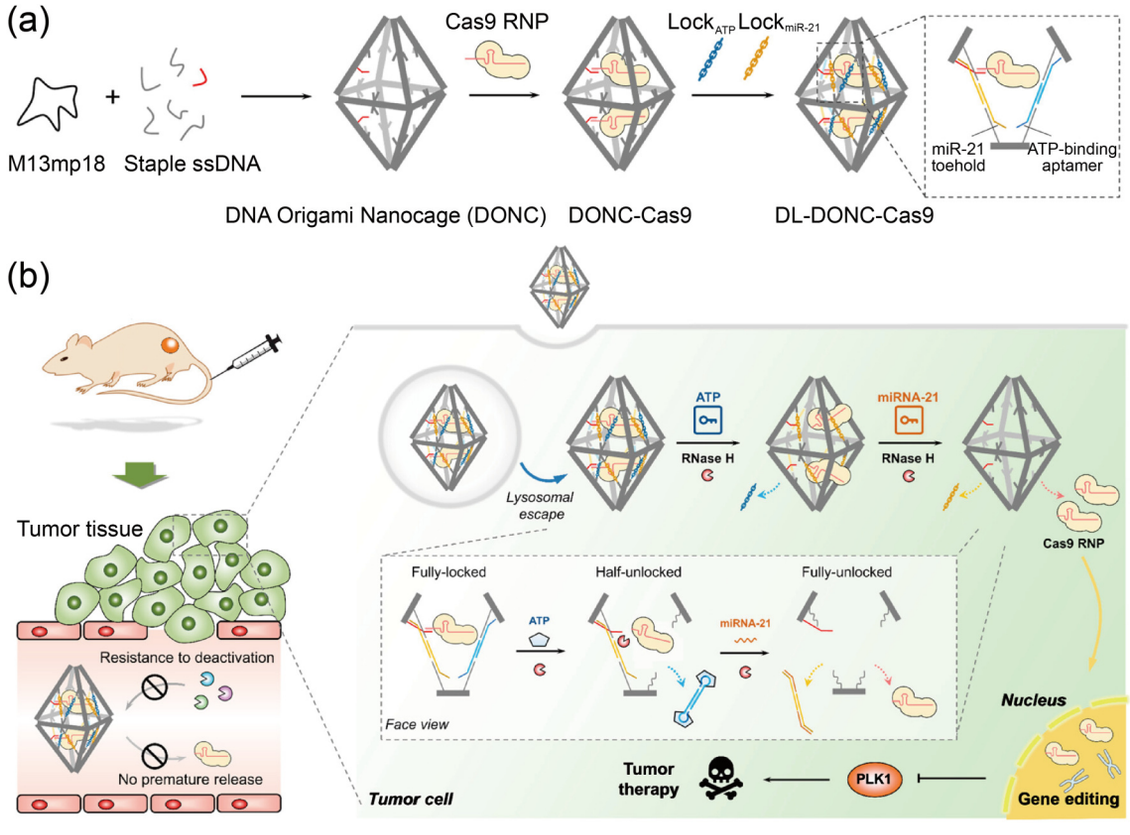

Fig. 10 (a) Construction of dual-chain-locked DNA origami nanocage for delivery of sgRNA/Cas9 ribonucleoprotein. **(b)** ATP and miR-21 responsive release of sgRNA/Cas9 ribonucleoprotein in tumor cells. Reproduced with permission from Ref. [103] © 2023 American Chemical Society.

Table 1 Representative DNA nanocarriers for delivery of sgRNA/Cas9 ribonucleoprotein

DNA nanocarriers	Targeting	Escape	Release	Ref.
RCA-based structure	—	PEI	—	[82]
	Aptamer	Mg^{2+}	miR-21	[83]
	—	—	Hha I	[84]
Branched DNA	—	—	DNase I	[87]
	Aptamer	HA peptide	RNase H	[88]
	—	—	RNase H	[89]
DNA origami	Aptamer	HA peptide	RNase H	[102]
	—	—	RNase H	[103]

nanocarriers for delivery of sgRNA/Cas9 ribonucleoprotein were summarized with the details of targeting strategy, escape mechanism, and release behavior.

Summary and Outlook

In this review, we summarized the recent advances in rationally designed DNA nanocarriers for delivery of sgRNA/Cas9 ribonucleoprotein. Various multifunctional DNA nanocarriers, including RCA-based DNA nanostructures, branched DNA, and DNA origami, have been precisely constructed and highlighted. These DNA nanostructures with excellent structural programmability and spatial addressability have been developed as versatile delivery vehicles for the homologous nucleic acid drugs. The sgRNA/Cas9 ribonucleoprotein as an RNA/protein complex can be efficiently loaded in these DNA nanocarriers through RNA/DNA hybridization. Furthermore, the active targeting groups and stimuli-responsive components can be site-specifically introduced into the DNA nanocarriers for targeted delivery and controlled release of the loaded sgRNA/Cas9 ribonucleoprotein *in vitro* and *in vivo*. This DNA nanostructure-based drug delivery system has played a significant role in achieving an enhanced targeted therapy and a reduced systemic toxicity during the treatments.

Meanwhile, some critical issues still should be properly addressed in the practical application of these DNA nanocarriers for delivering the sgRNA/Cas9 ribonucleoprotein in the clinic. First, the detailed understanding of the cellular internalization mechanism and intracellular fate of these DNA nanocarriers with different shapes and sizes should be further carefully investigated. Efficient package and delivery of sgRNA/Cas9 ribonucleoprotein into the target cells and nuclei is the first key step for effective gene editing. Second, the systemic pharmacokinetics of DNA nanocarriers, including distribution, metabolism, and excretion should be evaluated in detail. Third, off-target effect should be further deliberated to reduce the possible systemic toxicity during the treatments. Finally, the possible immune response induced by the exogenous DNA nanostructures should also be taken into consideration. Fortunately, the immunogenicity of DNA nanostructures can be highly reduced by careful sequence design and chemical modifications.

Despite the issues mentioned above, these DNA nanocarriers are still regarded as the promising delivery vehicles of gene editing system to edit and repair multiple genetic disease-related genes for the treatment of genetic disorders. We envision that pre-clinical and clinical studies based on the multifunctional DNA nanocarriers will emerge in the near future to achieve an efficient gene editing.

CRediT Author Statement

Hanyin Zhu，Jing Fan: Literature search, writing original draft. **Changping Yang:** Writing–review & editing. **Jianbing Liu:** Conceptualization, writing–review & editing. **Baoquan Ding:** Conceptualization, writing–review & editing.

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Conflict of Interests

The authors declare that no competing interest exists.

References

[1] J.D. Watson, F.H.C. Crick. Molecular structure of nucleic acids: A structure for deoxyribose nucleic acid. *Nature*, 1953, 171(4356): 737−738. https://doi.org/10.1038/171737a0

[2] F. Crick. Central dogma of molecular biology. *Nature*, 1970, 227(5258): 561−563. https://doi.org/10.1038/227561a0

[3] N.C. Seeman. Nucleic acid junctions and lattices. *Journal of Theoretical Biology*, 1982, 99(2): 237−247. https://doi.org/10.1016/0022-5193(82)90002-9

[4] M.M. Ali, F. Li, Z.Q. Zhang, et al. Rolling circle amplification: A versatile tool for chemical biology, materials science and medicine. *Chemical Society Reviews*, 2014, 43(10): 3324. https://doi.org/10.1039/c3cs60439j

[5] L.H. Eckardt, K. Naumann, W. Matthias Pankau, et al. Chemical copying of connectivity. *Nature*, 2002, 420(6913): 286. https://doi.org/10.1038/420286a

[6] P.W.K. Rothemund. Folding DNA to create nanoscale shapes and patterns. *Nature*, 2006, 440(7082): 297−302. https://doi.org/10.1038/nature04586

[7] N.R. Kallenbach, R.I. Ma, N.C. Seeman. An immobile nucleic acid junction constructed from oligonucleotides. *Nature*, 1983, 305(5937): 829−831. https://doi.org/10.1038/305829a0

[8] Y.G. Li, Y.D. Tseng, S.Y. Kwon, et al. Controlled assembly of dendrimer-like DNA. *Nature Materials*, 2004, 3(1): 38−42. https://doi.org/10.1038/nmat1045

[9] E.J. Cheng, Y.Z. Xing, P. Chen, et al. A pH-triggered, fast-responding DNA hydrogel. *Angewandte Chemie International Edition*, 2009, 48(41): 7660−7663. https://doi.org/10.1002/anie.200902538

[10] M.R. Jones, N.C. Seeman, C.A. Mirkin. Programmable materials and the nature of the DNA bond. *Science*, 2015, 347(6224): e1260901. https://doi.org/10.1126/science.1260901

[11] N.C. Seeman, H.F. Sleiman. DNA nanotechnology. *Nature Reviews Materials*, 2017, 3: 17068. https://doi.org/10.1038/natrevmats.2017.68

[12] X. Wang, C A.R. handrasekaran, Z.Y. Shen, et al. Paranemic crossover DNA: There and back again. *Chemical Reviews*, 2019, 119(10): 6273−6289. https://doi.org/10.1021/acs.chemrev.8b00207

[13] J. Chen, N.C. Seeman. Synthesis from DNA of a molecule with the connectivity of a cube. *Nature*, 1991, 350(6319): 631−633. https://doi.org/10.1038/350631a0

[14] R.P. Goodman, I.A.T. Schaap, C.F. Tardin, et al. Rapid chiral assembly of rigid DNA building blocks for molecular nanofabrication. *Science*, 2005, 310(5754): 1661−1665. https://doi.org/10.1126/science.1120367

[15] Y. He, T. Ye, M. Su, et al. Hierarchical self-assembly of DNA into symmetric supramolecular polyhedra. *Nature*, 2008, 452(7184): 198−201. https://doi.org/10.1038/nature06597

[16] C.X. Lin, M.Y. Xie, J.J.L. Chen, et al. Rolling-circle amplification of a DNA nanojunction. *Angewandte Chemie International Edition*, 2006, 45(45): 7537−7539. https://doi.org/10.1002/anie.200602113

[17] C.X. Lin, X. Wang, Y. Liu, et al. Rolling circle enzymatic replication of a complex multi-crossover DNA nanostructure. *Journal of the American Chemical Society*, 2007, 129(46): 14475−14481. https://doi.org/10.1021/ja0760980

[18] J.B. Lee, S.M. Peng, D.Y. Yang, et al. A mechanical metamaterial made from a DNA hydrogel. *Nature Nanotechnology*, 2012, 7(12): 816−820. https://doi.org/10.1038/nnano.2012.211

[19] C. Yao, H. Tang, W.J. Wu, et al. Double rolling circle amplification generates physically cross-linked DNA network for stem cell fishing. *Journal of the American Chemical Society*, 2020, 142(7): 3422−3429. https://doi.org/10.1021/jacs.9b11001

[20] M. Scheffler, A. Dorenbeck, S. Jordan, et al. Self-assembly of trisoligonucleotidyls: The case for nano-acetylene and nano-cyclobutadiene. *Angewandte Chemie International Edition*, 1999, 38(22): 3311−3315. https://doi.org/10.1002/(sici)1521-3773(19991115)38:22<3311::aid-anie3311>3.0.co;2-2

[21] F.A. Aldaye, H.F. Sleiman. Guest-mediated access to a single DNA nanostructure from a library of multiple assemblies. *Journal of the American Chemical Society*, 2007, 129(33): 10070−10071. https://doi.org/10.1021/ja073305n

[22] F.A. Aldaye, H.F. Sleiman, Modular access to structurally switchable 3D discrete DNA assemblies. *Journal of the American Chemical Society*, 2007, 129(44): 13376−13377. https://doi.org/10.1021/ja075966q

[23] B.R. Stepp, J.M. Gibbs-Davis, D.L.F. Koh, et al. Cooperative melting in caged dimers of rigid small molecule-DNA hybrids. *Journal of the American Chemical Society*, 2008, 130(30): 9628−9629. https://doi.org/10.1021/ja801572n

[24] J. Zimmermann, M.P.J. Cebulla, S. Mönninghoff, et al. Self-assembly of a DNA dodecahedron from 20 trisoligonucleotides with C_{3h} linkers. *Angewandte Chemie International Edition*, 2008, 47(19): 3626−3630. https://doi.org/10.1002/anie.200702682

[25] H. Yang, C.K. McLaughlin, F.A. Aldaye, et al. Metal–nucleic acid cages. *Nature Chemistry*, 2009, 1(5): 390−396. https://doi.org/10.1038/nchem.290

[26] P.K. Lo, P. Karam, F.A. Aldaye, et al. Loading and selective release of cargo in DNA nanotubes with longitudinal variation. *Nature Chemistry*, 2010, 2(4): 319−328. https://doi.org/10.1038/nchem.575

[27] M. Madsen, K.V. Gothelf. Chemistries for DNA nanotechnology. *Chemical Reviews*, 2019, 119(10): 6384−6458. https://doi.org/10.1021/acs.chemrev.8b00570

[28] Y.H. Dong, C. Yao, Y. Zhu, et al. DNA functional materials assembled from branched DNA: Design, synthesis, and applications. *Chemical Reviews*, 2020, 120(17): 9420−9481. https://doi.org/10.1021/acs.chemrev.0c00294

[29] E.S. Andersen, M.D. Dong, M.M. Nielsen, et al. Self-assembly of a nanoscale DNA box with a controllable lid. *Nature*, 2009, 459(7243): 73−76. https://doi.org/10.1038/nature07971

[30] S.M. Douglas, H. Dietz, T. Liedl, et al. Self-assembly of DNA into nanoscale three-dimensional shapes. *Nature*,

2009, 459(7245): 414−418. https://doi.org/10.1038/nature08016

[31] D.R. Han, S. Pal, J. Nangreave, et al. DNA origami with complex curvatures in three-dimensional space. *Science*, 2011, 332(6027): 342−346. https://doi.org/10.1126/science.1202998

[32] E. Benson, A. Mohammed, J. Gardell, et al. DNA rendering of polyhedral meshes at the nanoscale. *Nature*, 2015, 523(7561): 441−444. https://doi.org/10.1038/nature14586

[33] T. Gerling, K.F. Wagenbauer, A.M. Neuner, et al. Dynamic DNA devices and assemblies formed by shape-complementary, non–base pairing 3D components. *Science*, 2015, 347(6229): 1446−1452. https://doi.org/10.1126/science.aaa5372

[34] C.M. Huang, A. Kucinic, J.A. Johnson, et al. Integrated computer-aided engineering and design for DNA assemblies. *Nature Materials*, 2021, 20(9): 1264−1271. https://doi.org/10.1038/s41563-021-00978-5

[35] M. Kim, C. Lee, K. Jeon, et al. Harnessing a paper-folding mechanism for reconfigurable DNA origami. *Nature*, 2023, 619(7968): 78−86. https://doi.org/10.1038/s41586-023-06181-7

[36] A. Kuzuya, Y. Sakai, T. Yamazaki, et al. Nanomechanical DNA origami 'single-molecule beacons' directly imaged by atomic force microscopy. *Nature Communications*, 2011, 2: 449. https://doi.org/10.1038/ncomms1452

[37] H. Lee, A.K.R. Lytton-Jean, Y. Chen, et al. Molecularly self-assembled nucleic acid nanoparticles for targeted *in vivo* siRNA delivery. *Nature Nanotechnology*, 2012, 7(6): 389−393. https://doi.org/10.1038/nnano.2012.73

[38] H. Liang, X.B. Zhang, Y.F. Lv, et al Functional DNA-containing nanomaterials: Cellular applications in biosensing, imaging, and targeted therapy. *Accounts of Chemical Research*, 2014, 47(6): 1891−1901. https://doi.org/10.1021/ar500078f

[39] S. Saha, V. Prakash, S. Halder, et al. A pH-independent DNA nanodevice for quantifying chloride transport in organelles of living cells. *Nature Nanotechnology*, 2015, 10(7): 645−651. https://doi.org/10.1038/nnano.2015.130

[40] T.G.W. Edwardson, K.L. Lau, D. Bousmail, et al. Transfer of molecular recognition information from DNA nanostructures to gold nanoparticles. *Nature Chemistry*, 2016, 8(2): 162−170. https://doi.org/10.1038/nchem.2420

[41] G.L.Ke, M.H. Liu, S.X. Jiang, et al. Directional regulation of enzyme pathways through the control of substrate channeling on a DNA origami scaffold. *Angewandte Chemie International Edition*, 2016, 55(26): 7483−7486. https://doi.org/10.1002/anie.201603183

[42] P.C. Nickels, B. Wünsch, P. Holzmeister, et al. Molecular force spectroscopy with a DNA origami–based nanoscopic force clamp. *Science*, 2016, 354(6310): 305−307. https://doi.org/10.1126/science.aah5974

[43] Q.B. Mou, Y. Ma, G.F. Pan, et al. DNA Trojan horses: Self-assembled floxuridine-containing DNA polyhedra for cancer therapy. *Angewandte Chemie International Edition*, 2017, 56(41): 12528−12532. https://doi.org/10.1002/anie.201706301

[44] A.J. Thubagere, W. Li, R.F. Johnson, et al. A cargo-sorting DNA robot. *Science*, 2017, 357(6356): eaan6558. https://doi.org/10.1126/science.aan6558

[45] E. Kopperger, J. List, S. Madhira, et al. A self-assembled nanoscale robotic arm controlled by electric fields. *Science*, 2018, 359(6373): 296−301. https://doi.org/10.1126/science.aao4284

[46] X.G. Liu, F. Zhang, X.X. Jing, et al. Complex silica composite nanomaterials templated with DNA origami. *Nature*, 2018, 559(7715): 593−598. https://doi.org/10.1038/s41586-018-0332-7

[47] Q.Q. Hu, H. Li, L.H. Wang, et al. DNA nanotechnology-enabled drug delivery systems. *Chemical Reviews*, 2019, 119(10): 6459−6506. https://doi.org/10.1021/acs.chemrev.7b00663

[48] J. Zhang, Y.Y. Guo, F. Ding, et al. A camptothecin-grafted DNA tetrahedron as a precise nanomedicine to inhibit tumor growth. *Angewandte Chemie International Edition*, 2019, 58(39): 13794−13798. https://doi.org/10.1002/anie.201907380

[49] Y. Liu, J. Cheng, S.S. Fan, et al. Modular reconfigurable DNA origami: From two-dimensional to three-dimensional structures. *Angewandte Chemie International Edition*, 2020, 59(51): 23277−23282. https://doi.org/10.1002/anie.202010433

[50] P.S. Kwon, S.K. Ren, S.J. Kwon, et al. Designer DNA architecture offers precise and multivalent spatial pattern-recognition for viral sensing and inhibition. *Nature Chemistry*, 2020, 12(1): 26−35. https://doi.org/10.1038/s41557-019-0369-8

[51] H. Bila, K. Paloja, V. Caroprese, et al. Multivalent pattern recognition through control of nano-spacing in low-valency super-selective materials. *Journal of the American Chemical Society*, 2022, 144(47): 21576−21586. https://doi.org/10.1021/jacs.2c08529

[52] R. Ibusuki, T. Morishita, A. Furuta, et al. Programmable molecular transport achieved by engineering protein motors to move on DNA nanotubes. *Science*, 2022, 375(6585): 1159−1164. https://doi.org/10.1126/science.abj5170

[53] H. Lv, N.L. Xie, M.Q. Li, et al. DNA-based programmable gate arrays for general-purpose DNA computing. *Nature*, 2023, 622(7982): 292−300. https://doi.org/10.1038/s41586-023-06484-9

[54] J. Huang, A. Jaekel, J. van den Boom, et al. A modular DNA origami nanocompartment for engineering a cell-free, protein unfolding and degradation pathway. *Nature Nanotechnology*, 2024. https://doi.org/10.1038/s41565-024-01738-7

[55] J.L. Jiang, X.Y. Cui, Y.X. Huang, et al. Advances and prospects in integrated nano-oncology. *Nano Biomedicine and Engineering*, 2024, 16(2): 152−187. https://doi.org/10.26599/nbe.2024.9290060

[56] Y.R. Yu, D. Chen, Y.B. Yang, et al. Recent progress in electrochemical biosensors based on DNA-functionalized nanomaterials. *Nano Biomedicine and Engineering*, 2024. https://doi.org/10.26599/nbe.2024.9290071

[57] E. Deltcheva, K. Chylinski, C.M. Sharma, et al. CRISPR RNA maturation by trans-encoded small RNA and host factor RNase III. *Nature*, 2011, 471(7340): 602−607. https://doi.org/10.1038/nature09886

[58] G. Gasiunas, R. Barrangou, P. Horvath, et al. Cas9−crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, 2012, 109(39): E2579−E2586. https://doi.org/10.1073/pnas.1208507109

[59] M. Jinek, K. Chylinski, I. Fonfara, et al. A programmable dual-RNA–guided DNA endonuclease in adaptive bacterial immunity. *Science*, 2012, 337(6096): 816−821. https://doi.org/10.1126/science.1225829

[60] B. Wiedenheft, S.H. Sternberg, J.A. Doudna. RNA-guided genetic silencing systems in bacteria and Archaea. *Nature*, 2012, 482(7385): 331−338. https://doi.org/10.1038/nature10886

[61] L. Cong, F.A. Ran, D. Cox, et al. Multiplex genome engineering using CRISPR/cas systems. *Science*, 2013,

339(6121): 819−823. https://doi.org/10.1126/science.1231143
[62] P. Mali, L.H. Yang, K.M. Esvelt, et al. RNA-guided human genome engineering via Cas9. *Science*, 2013, 339(6121): 823−826. https://doi.org/10.1126/science.1232033
[63] J.A. Doudna, E. Charpentier. The new frontier of genome engineering with CRISPR-Cas9. *Science*, 2014, 346(6213): e1258096. https://doi.org/10.1126/science.1258096
[64] M. Jinek, F.G. Jiang, D.W. Taylor, et al. Structures of Cas9 endonucleases reveal RNA-mediated conformational activation. *Science*, 2014, 343(6176): e1247997. https://doi.org/10.1126/science.1247997
[65] D.B.T. Cox, R.J. Platt, F. Zhang. Therapeutic genome editing: Prospects and challenges. *Nature Medicine*, 2015, 21(2): 121−131. https://doi.org/10.1038/nm.3793
[66] G.J. Knott, J.A. Doudna. CRISPR-Cas guides the future of genetic engineering. *Science*, 2018, 361(6405): 866−869. https://doi.org/10.1126/science.aat5011
[67] X. Gao, Y. Tao, V. Lamas, et al. Treatment of autosomal dominant hearing loss by *in vivo* delivery of genome editing agents. *Nature*, 2018, 553(7687): 217−221. https://doi.org/10.1038/nature25164
[68] M. Wang, Z.A. Glass, Q. Xu. Non-viral delivery of genome-editing nucleases for gene therapy. *Gene Therapy*, 2017, 24(3): 144−150. https://doi.org/10.1038/gt.2016.72
[69] S.K. Alsaiari, S. Patil, M. Alyami, et al. Endosomal escape and delivery of CRISPR/Cas9 genome editing machinery enabled by nanoscale zeolitic imidazolate framework. *Journal of the American Chemical Society*, 2018, 140(1): 143−146. https://doi.org/10.1021/jacs.7b11754
[70] L. Li, S. Hu, X.Y. Chen. Non-viral delivery systems for CRISPR/Cas9-based genome editing: Challenges and opportunities. *Biomaterials*, 2018, 171: 207−218. https://doi.org/10.1016/j.biomaterials.2018.04.031
[71] W.H. Zhou, H.D. Cui, L.M. Ying, et al. Enhanced cytosolic delivery and release of CRISPR/Cas9 by black phosphorus nanosheets for genome editing. *Angewandte Chemie International Edition*, 2018, 57(32): 10268−10272. https://doi.org/10.1002/anie.201806941
[72] G.J. Chen, A.A. Abdeen, Y.Y. Wang, et al. A biodegradable nanocapsule delivers a Cas9 ribonucleoprotein complex for *in vivo* genome editing. *Nature Nanotechnology*, 2019, 14(10): 974−980. https://doi.org/10.1038/s41565-019-0539-2
[73] Y.C. Pan, J.J. Yang, X.W. Luan, et al. Near-infrared upconversion–activated CRISPR-Cas9 system: A remote-controlled gene editing platform. *Science Advances*, 2019, 5(4): eaav7199. https://doi.org/10.1126/sciadv.aav7199
[74] X.T. Yang, Q. Tang, Y. Jiang, et al. Nanoscale ATP-responsive zeolitic imidazole framework-90 as a general platform for cytosolic protein delivery and genome editing. *Journal of the American Chemical Society*, 2019, 141(9): 3782−3786. https://doi.org/10.1021/jacs.8b11996
[75] Q. Cheng, T. Wei, L. Farbiak, et al. Selective organ targeting (SORT) nanoparticles for tissue-specific mRNA delivery and CRISPR–Cas gene editing. *Nature Nanotechnology*, 2020, 15(4): 313−320. https://doi.org/10.1038/s41565-020-0669-6
[76] J.L. Zhuang, J.Z. Tan, C.L. Wu, et al. Extracellular vesicles engineered with valency-controlled DNA nanostructures deliver CRISPR/Cas9 system for gene therapy. *Nucleic Acids Research*, 2020, 48(16): 8870−8882. https://doi.org/10.1093/nar/gkaa683
[77] W.Q. Cai, T.L. Luo, L.Q. Mao, et al. Spatiotemporal delivery of CRISPR/Cas9 genome editing machinery using stimuli-responsive vehicles. *Angewandte Chemie International Edition*, 2021, 60(16): 8596−8606. https://doi.org/10.1002/anie.202005644
[78] T. Wan, J.F. Zhong, Q. Pan, et al. Exosome-mediated delivery of Cas9 ribonucleoprotein complexes for tissue-specific gene therapy of liver diseases. *Science Advances*, 2022, 8(37): eabp9435. https://doi.org/10.1126/sciadv.abp9435
[79] Y. Zou, X.H. Sun, Q.S. Yang, et al. Blood-brain barrier–penetrating single CRISPR-Cas9 nanocapsules for effective and safe glioblastoma gene therapy. *Science Advances*, 2022, 8(16): eabm8011. https://doi.org/10.1126/sciadv.abm8011
[80] G.Z. Zhu, R. Hu, Z.L. Zhao, et al. Noncanonical self-assembly of multifunctional DNA nanoflowers for biomedical applications. *Journal of the American Chemical Society*, 2013, 135(44): 16438−16445. https://doi.org/10.1021/ja406115e
[81] W.J. Sun, J.Q. Wang, Q.Y. Hu, et al. CRISPR-Cas12a delivery by DNA-mediated bioresponsive editing for cholesterol regulation. *Science Advances*, 2020, 6(21): eaba2983. https://doi.org/10.1126/sciadv.aba2983
[82] W.J. Sun, W.Y. Ji, J.M. Hall, et al. Self-assembled DNA nanoclews for the efficient delivery of CRISPR–Cas9 for genome editing. *Angewandte Chemie International Edition*, 2015, 54(41): 12029−12033. https://doi.org/10.1002/anie.201506030
[83] J.J. Shi, X. Yang, Y.N. Li, et al. MicroRNA-responsive release of Cas9/sgRNA from DNA nanoflower for cytosolic protein delivery and enhanced genome editing. *Biomaterials*, 2020, 256: 120221. https://doi.org/10.1016/j.biomaterials.2020.120221
[84] F. Li, N.C. Song, Y.H. Dong, et al. A proton-activatable DNA-based nanosystem enables co-delivery of CRISPR/Cas9 and DNAzyme for combined gene therapy. *Angewandte Chemie International Edition*, 2022, 61(9): 2116569. https://doi.org/10.1002/anie.202116569
[85] J.B. Liu, R.Y. Wang, D.J. Ma, et al. Efficient construction of stable gene nanoparticles through polymerase chain reaction with flexible branched primers for gene delivery. *Chemical Communications*, 2015, 51(44): 9208−9211. https://doi.org/10.1039/c5cc01788b
[86] F. Ding, Q.B. Mou, Y. Ma, et al. A crosslinked nucleic acid nanogel for effective siRNA delivery and antitumor therapy. *Angewandte Chemie International Edition*, 2018, 57(12): 3064−3068. https://doi.org/10.1002/anie.201711242
[87] F. Ding, X.G. Huang, X.H. Gao, et al. A non-cationic nucleic acid nanogel for the delivery of the CRISPR/Cas9 gene editing tool. *Nanoscale*, 2019, 11(37): 17211−17215. https://doi.org/10.1039/c9nr05233j
[88] J.B. Liu, T.T. Wu, X.H. Lu, et al. A self-assembled platform based on branched DNA for sgRNA/Cas9/antisense delivery. *Journal of the American Chemical Society*, 2019, 141(48): 19032–19037. https://doi.org/10.1021/jacs.9b09043
[89] N.C. Song, Y.W. Chu, S. Li, et al. Cascade dynamic assembly/disassembly of DNA nanoframework enabling the controlled delivery of CRISPR-Cas9 system. *Science Advances*, 2023, 9(35): eadi3602. https://doi.org/10.1126/sciadv.adi3602
[90] V.J. Schüller, S. Heidegger, N. Sandholzer, et al. Cellular immunostimulation by CpG-sequence-coated DNA origami structures. *ACS Nano*, 2011, 5(12): 9696−9702. https://doi.org/10.1021/nn203161y
[91] S.M. Douglas, I. Bachelet, G.M. Church. A logic-gated

nanorobot for targeted transport of molecular payloads. *Science*, 2012, 335(6070): 831−834. https://doi.org/10.1126/science.1214081

[92] Q. Jiang, C. Song, J. Nangreave, et al. DNA origami as a carrier for circumvention of drug resistance. *Journal of the American Chemical Society*, 2012, 134(32): 13396−13403. https://doi.org/10.1021/ja304263n

[93] Y.X. Zhao, A. Shaw, X.H. Zeng, et al. DNA origami delivery system for cancer therapy with tunable release properties. *ACS Nano*, 2012, 6(10): 8684−8691. https://doi.org/10.1021/nn3022662

[94] M.A. Rahman, P.F. Wang, Z.X. Zhao, et al. Systemic delivery of Bcl2-targeting siRNA by DNA nanoparticles suppresses cancer cell growth. *Angewandte Chemie International Edition*, 2017, 56(50): 16023−16027. https://doi.org/10.1002/anie.201709485

[95] S.P. Li, Q. Jiang, S.L. Liu, et al. A DNA nanorobot functions as a cancer therapeutic in response to a molecular trigger *in vivo*. *Nature Biotechnology*, 2018, 36(3): 258–264. https://doi.org/10.1038/nbt.4071

[96] R. Veneziano, T.J. Moyer, M.B. Stone, et al. Role of nanoscale antigen organization on B-cell activation probed using DNA origami. *Nature Nanotechnology*, 2020, 15(8): 716−723. https://doi.org/10.1038/s41565-020-0719-0

[97] S.L. Liu, Q. Jiang, X. Zhao, et al. A DNA nanodevice-based vaccine for cancer immunotherapy. *Nature Materials*, 2021, 20(3): 421−430. https://doi.org/10.1038/s41563-020-0793-6

[98] C. Sigl, E.M. Willner, W. Engelen, et al. Programmable icosahedral shell system for virus trapping. *Nature Materials*, 2021, 20(9): 1281−1289. https://doi.org/10.1038/s41563-021-01020-4

[99] X.H. Wu, C.P. Yang, H. Wang, et al. Genetically encoded DNA origami for gene therapy *in vivo*. *Journal of the American Chemical Society*, 2023, 145(16): 9343–9353. https://doi.org/10.1021/jacs.3c02756

[100] J. Yin, S.Y. Wang, J.H. Wang, et al. An intelligent DNA nanodevice for precision thrombolysis. *Nature Materials*, 2024, 23(6): 854−862. https://doi.org/10.1038/s41563-024-01826-y

[101] Y.C. Zeng, O.J. Young, C.M. Wintersinger, et al. Fine tuning of CpG spatial distribution with DNA origami for improved cancer vaccination. *Nature Nanotechnology*, 2024, 19(7): 1055−1065. https://doi.org/10.1038/s41565-024-01615-3

[102] W.T. Tang, T. Tong, H. Wang, et al. A DNA origami-based gene editing system for efficient gene therapy *in vivo*. *Angewandte Chemie International Edition*, 2023, 62(51): 2315093. https://doi.org/10.1002/anie.202315093

[103] Z.Q. Xu, Y.X. Dong, N.N. Ma, et al. Confinement in dual-chain-locked DNA origami nanocages programs marker-responsive delivery of CRISPR/Cas9 ribonucleoproteins. *Journal of the American Chemical Society*, 2023, 145(49): 26557−26568. https://doi.org/10.1021/jacs.3c04074