Review

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DNA-based nanostructures for RNA delivery

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Abstract: RNA-based therapeutics have emerged as a promising approach for the treatment of various diseases, including cancer, genetic disorders, and infectious diseases. However, the delivery of RNA molecules into target cells has been a major challenge due to their susceptibility to degradation and inefficient cellular uptake. To overcome these hurdles, DNA-based nano technology offers an unprecedented opportunity as a potential delivery platform for RNA therapeutics. Due to its excellent characteristics such as programmability and biocompatibility, these DNA-based nanostructures, composed of DNA molecules assembled into precise and programmable structures, have garnered significant attention as ideal building materials for protecting and delivering RNA payloads to the desired cellular destinations. In this review, we highlight the current progress in the design and application of three DNA-based nanostructures: DNA origami, lipid-nanoparticle (LNP) technology related to frame guided assembly (FGA), and DNA hydrogel for the delivery of RNA molecules. Their biomedical applications are briefly discussed and the challenges and future perspectives in this field are also highlighted.

Keywords: DNA origami; frame guided assembly (FGA); DNA hydrogel; RNA delivery; gene therapy; DNA-based nanostructures

Introduction

RNA-based therapy offers tremendous potential for the treatment of various diseases, including cancer, genetic disorders, and infections. Various types of RNA molecules, such as small interfering RNA (siRNA), microRNA (miRNA), messenger RNA (mRNA) as well as long non-coding RNA (LncRNA), can be harnessed for therapeutic purposes [1–3]. This innovative approach holds the promise of more precise and personalized treatments, and has attracted considerable interests from researchers, especially after the enormously successful application of mRNA vaccine in the COVID-19 pandemic. RNA therapeutics offer several advantages over traditional chemical medicines and protein therapeutics. They can target specific genes or proteins, resulting in fewer side effects and a reduced risk of off-target effects. Additionally, RNA therapeutics can be easily customized for rapid development and are more cost-effective to manufacture than protein therapeutics and have the potential for sustained and long-lasting effects [4]. Ongoing research in the field of RNA therapeutics is focused on overcoming challenges such as RNA stability, immune responses, and delivery efficiency particularly. There is an urgent need for the development of RNA delivery technology and suitable delivery carriers. Nowadays, the most used approaches for RNA delivery are based on viral and non-viral vectors. Viral vectors typically display a high degree of immunogenicity and mutagenicity, while non-viral vectors, including lipids, polymers, nucleic acid nanostructures, peptides, and virus-like particles are highly anticipated for low immunogenicity and a reduced risk of randomly integrating viral genetic material into the genome [5]. For example, the vesicles formed by lipid-nanoparticle (LNP) can protect nucleic acids from degradation, enhance delivery stability, and effectively encapsulate various nucleic acid substances including siRNA, mRNA, miRNA, etc. Although LNP technology is currently the mainstream carrier for RNA delivery, it is constrained by issues such as high cytotoxicity, poor storage stability, and serious immunogenicity. In contrast, DNA nanotechnology has emerged as a promising delivery tool, with many nucleic acids themselves having drug-forming properties that make them suitable for RNA delivery into organisms [6]. DNA nanostructures use hydrogen binding or molecular recognition instead of charging interactions to condense functional nucleic acids and form delivery complexes. Many DNA nanostructures have the ability to rapidly internalize into various cell lines through endocytosis without the need for transfection agents [7]. Additionally, DNA nanostructured carriers have

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the advantages of high biocompatibility, high controllability, low immunogenicity, effective drug loading, and prolonged circulation time *in vivo*, making them compelling candidates for RNA delivery. In this review, we briefly described the developments of three DNA nanotechnologies applied for RNA delivery, including DNA origami, LNP technology related to frame guided assembly (FGA) and DNA hydrogel. We also discussed potential challenges and strategies for the development of improved RNA delivery carriers. This progress in RNA-based therapy and the innovative approaches being developed for RNA delivery systems hold great promise for the future, offering potential breakthroughs in medical treatments and personalized medicine (Figure 1).

DNA origami

DNA origami, proposed by Rothemund in 2006, has revolutionized the field of DNA nanotechnology by simplifying the design of DNA nanostructures and expanding their potential applications [8]. The technique is based on the Watson-Crick principle, where a scaffold is folded into a pre-designed, predictable, addressable structure with various morphologies through several short staples. Unlike other assembly strategies for DNA nanostructures, DNA origami is less affected by chemometrics and the purity of oligonucleotide single strands. This allows for the production of uniform target nanostructures within a few hours using a simple annealing program under normal excessive staple strands conditions [9]. In recent years, with the development of DNA origami technology, the designed structures have evolved from one-dimensional and two-dimensional structures (squares, triangles, stars, smiling faces, etc.) to three-dimensional structures (boxes, bottles, tetrahedrons, etc.) [10]. Various design software has greatly facilitated researchers to design precise, larger, and more complex nanostructures. By inserting or deleting responsive base pairs at the designed positions, distorted and curved three-dimensional shapes can be designed to form nanostructures with complex curvature, such as mazes [11], polyhedral structures [12], multi-layer structures [13], wireframe structures [14], etc. In addition, the use of external support surfaces such as mica or lipid bilayers has facilitated the assembly of larger functional DNA origami [15, 16]. The implementation of modular composite assembly structures has also facilitated the construction of larger-scale nanostructures, further enhancing the potential of DNA origami technology [17].

Furthermore, the emergence of other new DNA origami methods has expanded the possibilities of DNA origami technology. Gothelf and his team reported the triplex origami that can compress and protect DNA from enzymatic degradation by triplex-forming oligonucleotides (TFOs). This method is compatible with Watson-Crick based methods and enables unprecedented spatial control over dsDNA templates. The relative rigidity of dsDNA in the triplex-forming DNA origami structure requires fewer starting materials, allowing for the formation of larger structures and lowers costs [18]. Another type of DNA origami is Meta-DNA, which mimics the self-assembly of short DNA strands to promote the modular assembly of DNA structures at the submicron or micron level [19]. Additionally, a biological strategy to overcome the bottleneck in the chemical synthesis of long single-stranded DNA (ssDNA) sequences involves the use of trans-cleavage DNA ribozymes. This approach allows for the efficient amplification and preparation of long ssDNA sequences through *in vitro* or *in vivo* rolling ring replication, followed by the spontaneous cleave and release ssDNA assembly sequences by DNA ribozymes [20]. Furthermore, the ongoing advancements in DNA origami detection technology will enhance our understanding of the self-assembly mechanism inherent in DNA origami structures [21], thereby laying a strong foundation for the potential application of DNA origami technology.

The continuous development of nucleic acid nanotechnology has led to the introduction of a wide range of modified functional groups aimed at enhancing the functionalization of origami structures. Taking advantage of the programmability and addressability of DNA origami, modified oligonucleotide staples can be integrated directly or through short strands extension hybridization after structural folding. By carefully adjusting parameters such as quantity, distance, and graphic position, the functionality and applicability of DNA origami structures can be expanded for various biomedical and nanotechnological applications. Furthermore, the combination of modified functional groups such as proteins [22, 23], nucleic acids [24, 25], liposomes [26, 27], small molecule drugs [28], chemical groups [27, 29–33], as well as assembly of inorganic materials (gold nanoparticles [34], carbon nanotubes [35], quantum dots [36, 37], etc.) has opened up new possibilities for functionalizing DNA origami structures for the targeted delivery of drugs or RNAs to biological systems. Table 1 provides a summary of the latest developments in DNA origami structures for RNA loading and release.

DNA origami as carrier for RNA delivery

The unique folding of DNA origami with specific structures and functions has opened up new possibilities for the design and fabrication of nanoscale devices for drug delivery and

Table 1: Examples of functional DNA origami for RNA delivery.

RNA delivered	Origami shapes	Responsive stimuli	Targets	References
siRNA	Tetrahedron	N/A	Firefly luciferase mRNA; GFP mRNA	[38]
siRNA	DNA cage	Cellular miRNA or mRNA	Fatty acid synthase (FASE) Bcl2 and Bcl-xL mRNA	[39]
siRNA	Rectangular, Tubular	N/A	Bcl2 mRNA	[40]
shRNA	Triangular	GSH	P-gp and surviving mRNA	[41]
siRNA	Tetrahedron	Ribonuclease H (RNase H)	EGFP mRNA	[42]
siRNA	DNA hairpin tile, monomer, tetrahedron, nanostring	N/A	mGFP5 mRNA	[43]
dsRNA	Tubular	pH	TLR	[44]
siRNA	Tubular	GSH	Bcl2 and P-gp mRNA	[45]
siRNA	Tetrahedron	N/A	Braf mRNA	[46]
siRNA	Octahedral	RNase	CTGF and HSP72 mRNA	[47]
miRNA	Tetrahedron	RNase H	miRNA	[48]
siRNA	Tetrahedron	pH	TNFα mRNA	[49]
miRNA	Tetrahedron	N/A	DKK1 mRNA	[50]
siRNA	Soccer-ball like	pH	Bcl2L-12 mRNA	[51]

N/A, Not Applicable; GFP, Green fluorescent protein; Bcl2, B cell lymphoma 2; Bcl-xL, Recombinant Human B-Cell Leukemia/Lymphoma XL; P-gp, P-glycoprotein; EGFP, Enhanced green fluorescent protein; mGFP5, monomeric enhanced green fluorescent protein 5; TLR, Toll-like receptor; CTGF, connective tissue growth factor; HSP72, heat shock protein 72; TNFα, tumor necrosis factor alpha; DKK1, Corticosteroids-induced Dickkopf-1; Bcl2L-12, B cell lymphoma 2 like protein 12.

also holds great potential for delivering therapeutic RNA molecules to target cells (Figure 1). DNA origami is considered a promising delivery tool due to its high programmability, precise addressability, high biocompatibility, low immunogenicity, and easy assembly. In recent years, researchers have conducted extensive cell delivery studies using DNA origami structures with various shapes, such as triangles, rectangles, rods, nanotubes, nanocages, tetrahedra, cubes, octahedra, icosahedra and more [23, 36, 38, 40, 41, 52–54]. The well-designed DNA origami nanostructures and modifications have been developed as carriers of RNA. Rahman et al. successfully developed DNA nanoparticles (DNPs) with

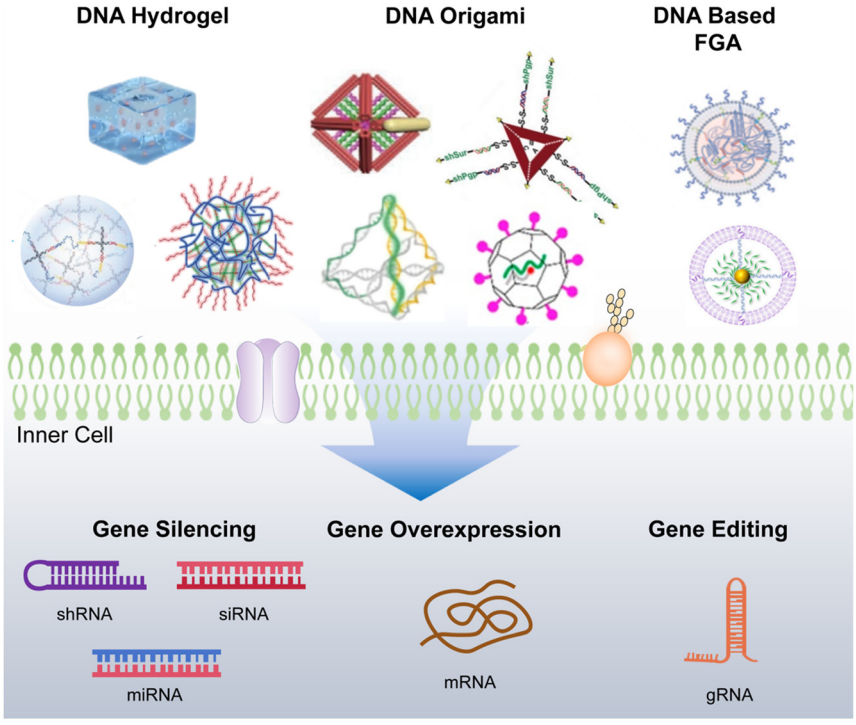

Figure 1: DNA-based nanostructures for RNA delivery. (This figure is reproduced with permission from Refs. [7, 41, 47, 49, 98]: Copyright 2018 or Copyright 2021–2022, Wiley: Hoboken, NJ, USA; from Ref. [91], Copyright 2023, Springer Nature; from Refs. [51, 81], Copyright 2023, American Chemical Society: Danvers, MA, USA; from Ref. [80], Copyright 2023, Royal Society of Chemistry: London, UK.).

rectangular or tubular shapes of varied dimensions using the modular DNA brick method. They loaded B cell lymphoma 2 (Bcl2) siRNAs through surface extended protruding single-stranded hybridization and investigated their cellular uptake in cancer cells. The results showed that the siRNA delivered by DNP inhibited cell growth *in vitro* and *in vivo*, suppressing tumor growth in xenograft models associated with Bcl2 deficiency (Figure 2A) [40]. In 2018, Ding Group designed a triangular DNA origami structure to serve as a co-delivery carrier for two different small hairpin RNA (shRNA) transcription templates targeting multidrug resistance (MDR) associated genes (gene of P-glycoprotein and survivin). This triangular DNA origami bearing cleavable disulfide capture strands on each side and targeting aptamers in each vertex (TO) was assembled in one pot reaction (Figure 2B) [41]. Zhang et al. reported the design of DNA hairpin tile monolayer, tetrahedron, nanostring and other engineering DNA nanostructures as carriers for delivering siRNA into plant cells, indicating the universality of DNA origami structure for delivering RNA drugs [43].

Compared to linear or planar nucleic acid frameworks, some three-dimensional framework nanostructures offer a unique ability to smoothly cross the cell membrane barrier through specific interactions with the cell surface. Among these structures, the tetrahedral DNA origami structure has garnered significant attention due to its excellent mechanical and biological properties, low immunogenicity, simple design process, easy fabrication, and high production yield. Thus, it has become widely used in the field of biomedicine and has shown promise in addressing challenges in RNA delivery. Lee et al. reported the design of self-assembled DNA tetrahedral nanoparticles with well-defined sizes to effectively deliver siRNAs into cells and silence target genes in tumors. The siRNA is anchored to tetrahedral edge by elongated sequences of designed nanostructures and self-assembled to the structure in one step. These nanoparticles exhibit prolonged blood circulation time for siRNA *in vivo* [38]. Nucleic acid nanostructures can enter cells through endocytic or non-endocytic pathways, which mainly include phagocytosis, grid protein dependent endocytosis, caveolin dependent endocytosis, and micropinocytosis. By adding inhibitors to block the pathway, the pathway of structure entry into cells can be explored [55]. Fan et al. reported that 3D tetrahedral DNA nanostructures (TDNs) can minimize electrostatic repulsion through the “angle attack” mode and induce uneven charge redistribution in the membrane, ultimately resulting in internalization via the caveolae-mediated endocytosis pathway [56]. More recently, Lin

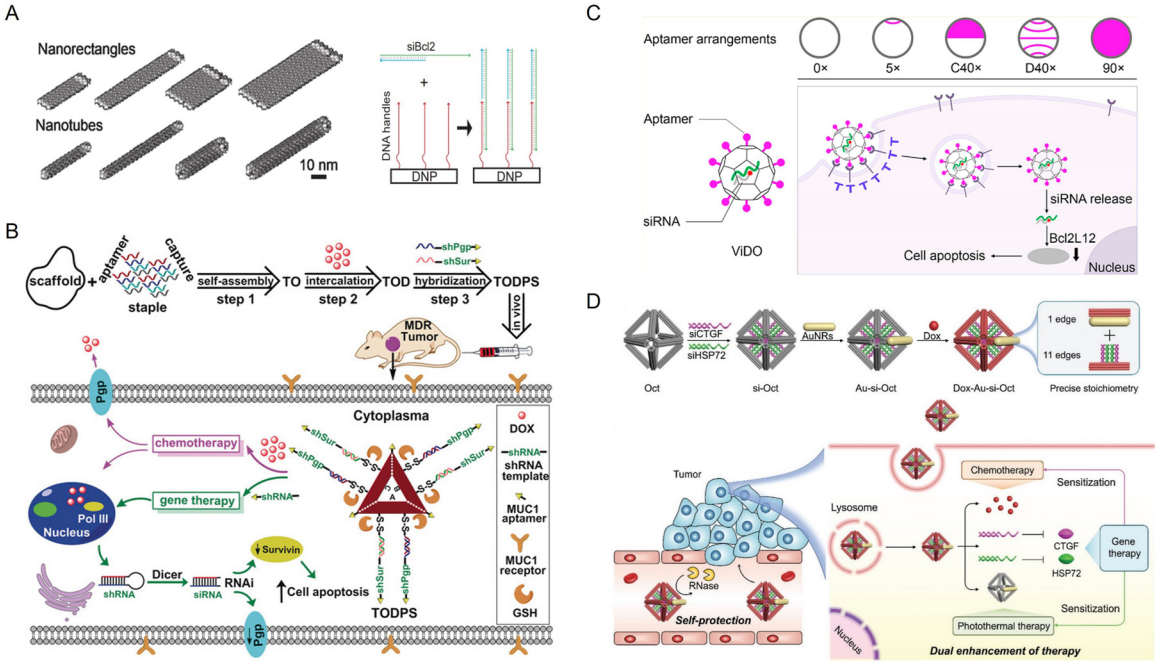

Figure 2: DNA origami as carrier for RNA delivery. (A) Delivery of Bc12-targeting siRNA by rectangular and tubular DNA origami nanostructures of different sizes. Figure reproduced with permission from Ref. [40], Copyright 2017, Wiley: Hoboken, NJ, USA; (B) A triangular DNA origami nanoplatform for synergistic treatment of multidrug-resistant tumors through a combination of RNA interference (RNAi) and chemotherapy. Figure reproduced with permission from Ref. [41], Copyright 2018, Wiley: Hoboken, NJ, USA; (C) A virus mimetic DNA origami carrier for siRNA delivery and modulated cell endocytosis and apoptosis. Figure reproduced with permission from Ref. [51], Copyright 2023, American Chemical Society: Danvers, MA, USA; (D) Octahedral DNA origami nanostructures enabled self-protective siRNA delivery for dual enhancement of chemo-photothermal combination therapy. Figure reproduced with permission from Ref. [47], Copyright 2021, Wiley: Hoboken, NJ, USA.

et al. utilized DNA tetrahedral structures as a delivery system for miRNA-mediated gene inhibition. By directly assembling miRNA sequences onto these tetrahedral structures through hybridization, researchers discovered that these structures had remarkable tissue penetration capabilities, enabling them to effectively traverse the skin barrier and reach deep into the dermis. Moreover, they demonstrated that the degree of penetration was influenced by the topological structure and size of the tetrahedron [48]. In addition to tetrahedral DNA nanostructures, there have been reports of delivery carriers with various other DNA origami nanostructures shapes, such as tubular [44], octahedral [47], soccer-ball shaped [51]. The wide range of DNA origami nanostructures provides a flexible and customizable platform for delivery systems, opening up new possibilities for RNA delivery applications.

There are diverse factors that affect the delivery properties of DNA nanostructures, including cell type, structural shape, size, rigidity, stability, and targeted molecular modification [52, 57–60]. Among them, targeted molecule modification, in particular, has been shown to improve the delivery efficacy. With the exceptional design flexibility of DNA nanostructures, researchers have been able to increase targeting activity of drug by incorporating modifications such as nucleic acid aptamers or targeted ligands on the structure. This strategy has also advanced the application of RNA delivery, with RNA loaded DNA origami being further enhanced by integration with chemotherapy drugs, nucleic acid aptamers and various functional groups. This integration synergistically increases the efficiency and versatility of these carriers, making them more effective in their targeted applications. Lee et al. demonstrated that the tetrahedral DNA origami structure can improve siRNA delivery by incorporating cancer-targeting ligands such as peptides and folic acid [38]. Subsequent studies have highlighted the importance of the spatial orientation and density of ligand modification. Optimal delivery occurs when at least three folic acid molecules are attached to each nanoparticle, and gene silencing is only observed when the ligand is in the appropriate spatial orientation. Similarly, Xiao et al. engineered the tetrahedral framework nucleic acids (tFNAs) by incorporating anti-Braf siRNA (siBraf) via sticky ends to knockdown the expression of target gene. They functionalized the structure by integrating the aptamer AS1411, which targets nucleolin on the surface of A375 cells. This modification resulted in a significantly improvement in cellular uptake efficiency and increased potency in cleaving Braf mRNA compared to free siBraf. This study presents an innovative approach in combining siRNA and aptamers using tFNA for gene silencing, offering a promising avenue in the field of gene therapy [46]. Moreover, unique structural designs, such as biomimetic structures, have demonstrated the capability to enhance the effectiveness of RNA delivery. By mimicking the size and shape as well as the gene-loading feature of viruses, Yang's team constructed a soccer-ball shaped virus-inspired DNA origami (ViDO) structure as a programmable siRNA carrier. In their design, siRNAs are loaded through complementary pairing with 90 anchor strands that extend toward inside the structure. The outer modification enables different numbers and arrangements of aptamers. They tested different distribution patterns of aptamers (AS1411, Sgc8) in the structure to modify the endocytosis and transfection efficiency of cell uptake. They found that an increased number of aptamers enhanced cellular uptake, and a dispersed distribution of aptamers with a medium number was more conducive to cell uptake (Figure 2C) [51]. Xu et al. presented a novel approach involving 40 nm-sized octahedral DNA origami frameworks (OctDOFs) with six helical edges for precise, coordinated delivery of siRNAs (siCTGF and siHSP72), chemo drugs (doxorubicin, Dox) and photodynamic agents (gold nanorods, AuNRs). siRNAs were site specifically anchored in the interior of OctDOFs via RNA-DNA hybridization. The rigid structure's inner cavity provided protection for the encapsulated siRNAs from RNase degradation and protein binding during transportation, thereby enabling effective downregulation of connective tissue growth factor (CTGF) and heat shock protein 72 (HSP72), and rendering cancer cells sensitive to chemo drugs and hyperthermia. This OctDOFs nano vehicle offers a promising platform for precise medication and synergistic treatment of drug-resistant cancer using siRNA delivery (Figure 2D) [47]. Li et al. developed a novel approach by integrating tetrahedral DNA nanostructures with an injectable heparin lithium hydrogel to synergistically deliver miR335-5p, a molecule that targets Dickkopf-1 (DKK1) and upregulates Wnt signaling pathway, for the purpose of bone regeneration and defect repair [50]. A sophisticated multifunctional nano-system for tumor treatment and diagnostics was reported by Luo et al. This system leverages a tetrahedral DNA origami structure that was modified with various component including miRNA (miR-21), aptamer (AS1411), two pairs of DNA/RNA hybrids, a pH-sensitive DNA catcher (i-motif), chemotherapy drug (Dox), and was labeled with Cy5/BHQ2 signal tags for miRNA fluorescence imaging and chemical/gene therapy [61].

Designable responsiveness for RNA release

DNA origami structures have achieved significant advancements in structural assembly not only in terms of structural

dimensions, topological weaving, structural curvature, modular assembly, but also in dynamic control such as DNA walker and DNA nano robot [62]. The application of DNA origami nanostructures has been revolutionized by the implementation of responsive design, which allows for the effective release of delivered RNA drugs. Tailored responsive designs can be utilized to optimize the release of delivered RNA drugs, utilizing a series of responsive moieties that can be triggered by glutathione (GSH), pH levels, and cellular enzymes (such as esterases and phosphatases) [63, 64] (Table 1). A tubular DNA nanostructure was engineered to carry siRNAs targeting Bcl2 and P-glycoprotein (P-gp) genes. Additionally, it was modified with a transcriptional trans-activating peptide (TAT), known for its ability to enhance cell uptake and improve tumor retention of the nanostructures. The nanostructure was also equipped with a disulfide-responsive DNA locking strand, designed to keep the nanostructure in a closed state. Upon exposure to reducing agents like glutathione (GSH) in the cytoplasm, the disulfide bond breaks, triggering the opening of the nanostructure and allowing for the release of siRNA and the chemotherapy drug Doxorubicin. This smart design enables targeted and controlled delivery of therapeutic agents in response to cellular redox environments (Figure 3A) [45]. Similarly, in the design of the triangular planar framework DNA origami nanocarrier reported by Liu et al., in addition to the co-delivery of two shRNAs and the chemotherapy drug Dox, the short aptamer of the MUC1 sequence was also modified at the end of the strand of the device for better targeting cancer cells, and at the same time, cleavable disulfide linkage with a redox-responsive property to GSH was also designed to control the release of shRNAs (Figure 2B) [41].

pH levels play a crucial role in many functional aspects of organisms. Unlike healthy tissues, which usually have a normal physiological pH around 7.4, regions affected by diseases such as tumors often exhibit abnormally high level of acidity [65, 66]. pH targeting can be developed as a valuable strategy for cancer treatment. The most commonly used functional units for pH sensing in cancer treatment are the i-motif and DNA triplet structure. Among these, the i-motif is a DNA quadruplex composed of four streets of cytosine-rich DNA sequences that come together to form C CH^+ base pairs. The stabilization of i-motif structures requires slightly acidic conditions, as first proposed by Liu et al. [67]. This structure can undergo rapid and highly reversible conformational transitions between the four chains, with i-motif structures forming at weakly acidic pH values (<6.0) and random curls at higher pH values (>6.4) [68]. For example, Gao et al. created a tailored tFNA nanobox capable of delivering siRNA in a pH-responsive manner. They designed the i-motif sequence at the apex of the DNA tetrahedral structure to achieve the release of siRNA in response to pH changes, allowing for controlled release upon entry into lysosomes. The tFNA nanobox demonstrated excellent biocompatibility and stability in serum, paving the way for its potential application as a safe and effective small RNA delivery vehicle for therapeutic purposes [49]. The pH responsiveness of DNA triplexes also provides a versatile tool for the development of pH-regulated systems. This involves the specific binding of auxiliary single strand, named triplex-forming oligonucleotide (TFO), to the major groove of duplex DNA through Hoogsteen or reverse Hoogsteen hydrogen interactions [69]. Under low pH conditions, the protonation of TFO strands lead to their non-covalent binding to the major groove of dsDNAs, forming triplex configurations like T·A-T and C+· G-C. This triplex configuration was dissociated into duplex and single-stranded TFO upon deprotonation under high pH [70]. Ding group has successfully developed a DNA nanodevice vaccine by meticulously assembling two types of toll-like receptor (TLR) agonists (dsRNA and CpG DNA) and an antigen peptide within the inner cavity of tubular DNA nanostructures. The integration of low pH responsive DNA locking strands, a type of triplex DNA, on the exterior of the nanostructure enables the vaccine to open in the lysosomes of antigen-presenting cells. Exposure to adjuvants and antigens triggers T cell activation and cancer cytotoxicity, leading to effective immune mediated tumor regression (Figure 3B) [44].

Drawing inspiration from the digestion property of RNase H, which cleaves the RNA strand of an RNA–DNA duplex, Lin et al. reported a novel bio-switchable miRNA inhibitor delivery system (BiRDS) constructed with three miRNA inhibitors and a nucleic acid core. The designed RNase H-responsive sequence, a type of DNA-RNA hybrid switch mentioned above, enables the structure to transition from a 3D structure to a 2D structure after entering cells, promoting the delivery of miRNA inhibitors to their target sites within the cells (Figure 3C) [48]. Moreover, Ruan et al. described the creation of a DNA nanostructure using DNA rolling circle amplification (RCA) that generated a clew-like shape structure (DNA nanoclew, DC). This innovative structure allowed for the attachment of multiple copies of siRNA duplexes through complementary overhangs of the DC linear template, resulting in the formation of a unique SNA nanoparticle. Subsequently, the siRNA could be released through cleavage by the intracellular Dicer enzyme [71].

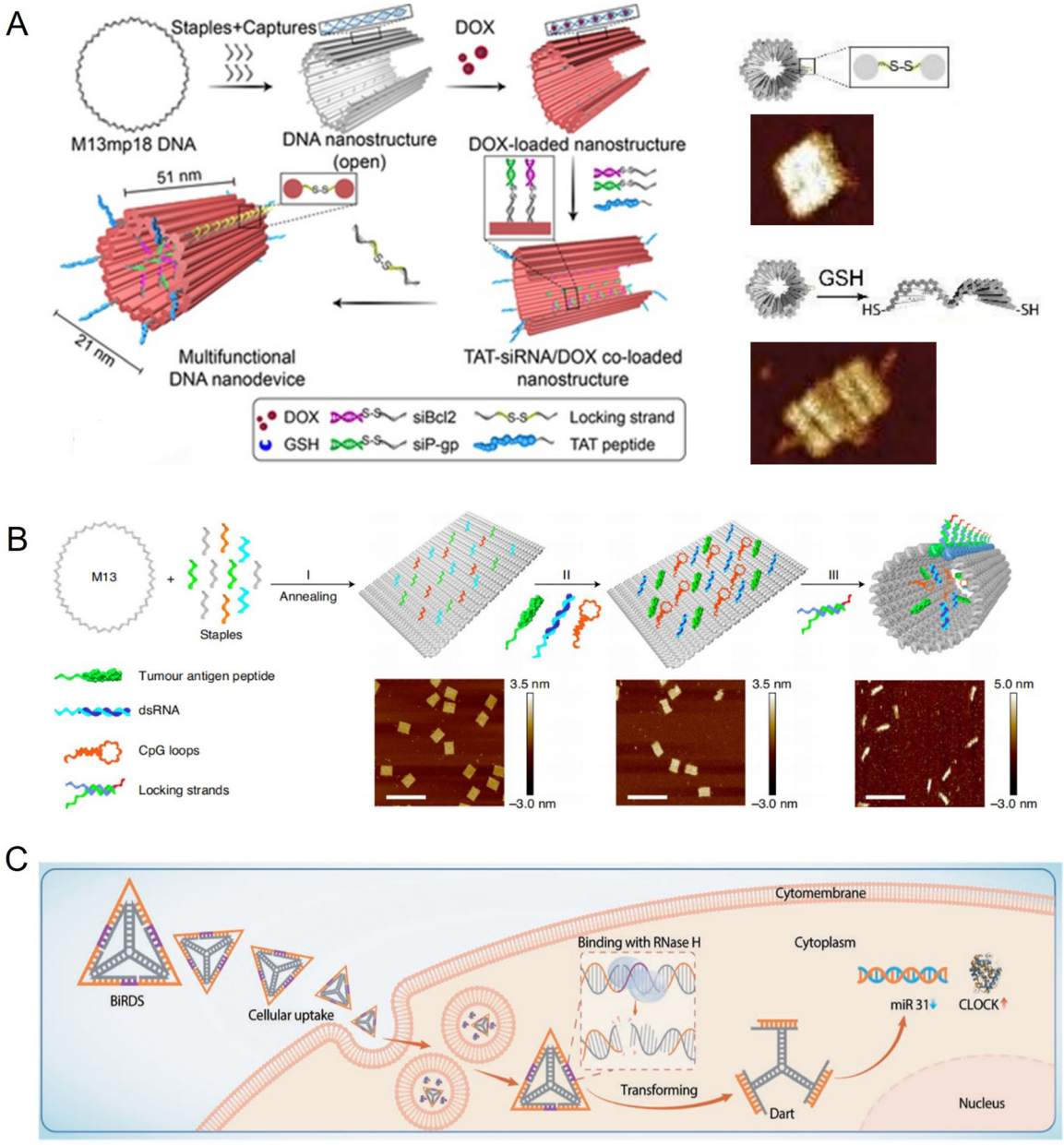

Figure 3: Designable responsiveness of DNA origami carrier release for RNA delivery. (A) A tubular DNA origami nanodevice vaccine with locking strand modifications for cancer immunotherapy. Figure reproduced with permission from Ref. [44], Copyright 2022, Springer Nature; (B) Tubular DNA nanodevice with disulfide bond modifications trigger opening and release in response to intracellular glutathione (GSH) as a siRNA/chemo-drug co-delivery vehicle for combined cancer therapy. Figure reproduced with permission from Ref. [45], Copyright 2020, Wiley: Hoboken, NJ, USA; (C) A tetrahedral DNA origami bio-switchable miRNA inhibitor delivery system. Figure reproduced with permission from Ref. [48], Copyright 2022, Wiley: Hoboken, NJ, USA.

RNA delivery of frame guided assembly (FGA) and applications

LNP, as a leading carrier technology, is widely utilized for RNA delivery. It typically involves the delivery of RNA by the electrostatic adsorption of positively charged liposomes with the negative charged nucleic acid, enabling the formation of nanoparticles through hydrophobic interactions [72]. However, this approach has several limitations. For example, changes in the electrostatic potential of LNP can cause premature separation and release of RNA, rendering it ineffective. Therefore, strict cold chain storage and transportation are required [73]. Additionally, lipid nanoparticles themselves

may stimulate immune responses due to factors such as surface charge, size of liposomes and the incorporation of polyethylene glycol (PEG) which affect the type and efficiency of immune reactions [74]. Positive surface charges may also cause cytotoxicity through damage to cell membranes or proteins [72]. Moreover, ionizable LNPs excipients also cause instability during long-term storage [75]. The size and morphology of nanoparticles play a crucial role in determining their biological cytotoxicity, and precise control of these factors is crucial for improving RNA targeting and therapeutic efficacy. Besides that, the internal organization of lipid nanoparticles remains an unsolved matter to date. Different LNP delivery can lead to different roles in the internal circulation, which in turn determines the different organ selectivity of LNP [76].

The ongoing optimization of LNP technology focuses on a variety of key goals, including minimizing potential immune responses, enhancing packaging efficiency, improving *in vivo* transfection effectiveness, extending survival times, ensuring stable storage, convenient transportation, facilitating targeted release, and streamlining cost-effective industrial production. These efforts are all aimed at maximizing the overall impact and utility of LNP technology. The use of frame-based methods to prepare nanostructures as carriers offers greater control over structure preparation and allows for extensive modifications to enhance delivery capabilities. For example, the development of spherical nucleic acid (SNA) nanostructures such as oligonucleotide-modified gold nanoparticles has shown more effectively deliver antisense oligodeoxynucleotides into cells for adjustable gene knockdown (Figure 4A) [77]. Multiple DNA conjugated gold nanoparticles (DNA-GNPs) have advantages such as sharp melting transitions on binding to complementary DNA, enhanced target binding affinity, high stability in high salt buffer, low/non-toxicity, resisting nuclease degradation, and effective uptake by many different cell lines. They are widely used in the fields of biological diagnosis and nanomedicine [66]. Lee et al. reported that siRNA was bound to hydrophilic polymer PEG modified gold nanoparticles through biodegradable disulfide bonds, and coated with a library of end modified poly (beta-amino ester)s (PBAEs), achieving good siRNA delivery effects (Figure 4B) [78].

The assembly of LNP-SNA structures based on LNP cores can be viewed as a framework that influences the assembly of liposomes into nanostructures. Research has shown that the uptake of LNPs is largely mediated by the LDL receptor, while SNA uptake is primarily mediated by class A scavenger receptors, which recognize DNA. When LNP-SNAs are injected intravenously, the encapsulated mRNA is primarily expressed in the spleen, whereas mRNA encapsulated by LNPs (without DNA on the surface) is mainly expressed in the liver [3]. In addition, virus inspired encapsulated DNA octahedral origami nanostructures reported as a form of frame-guided assembly (Figure 4C) [79]. Similarly, gold nanoparticles or other nanoparticles as elemental templates, followed by enhancing structural functions through corresponding modifications, many related reports have achieved ideal delivery [82, 83]. However, most of them still rely on liposome components such as cations or ionizable liposomes, or there is still a lack of comprehensive solutions in terms of drug protection and loading rate. The “frame guided assembly (FGA)” strategy proposed provides a new and promising assembly method.

The FGA strategy, a novel self-assembly method introduced by Liu Group, allows for the precise control over virous nanostructures. This approach serves as scaffolds for the preparation of controllable vesicles with programmable loading of various drug molecules as well as RNAs [84, 85]. The FGA strategy has demonstrated universal assembly capabilities, leading to the self-assembly of amphiphilic molecules in both planar and three-dimensional frameworks [86, 87]. In the application of RNA delivery, Liu’s group utilized FGA strategy to prepare a delivery system based on gold nanoparticles for the delivery of Bcl-2 ASO and siRNA (siEGFP), effectively inhibiting cancer cell growth (Figure 4D) [80]. Researchers found that a 20 % anchoring chains to assemble FGA structures for loading ASO result in the full assembly of ASO-loaded liposomes at low surfactant (Octyl β-D glucopyranoside, OG) concentration. In contrast, using 5 % (w/v) OG led to the formation of more incomplete vesicles or frame-coated structures. This indicates that the assembly of the structure is related to the concentration of surfactants. Additionally, siRNA was designed to be immobilized on AuNP and hybridized to introduce Y-shaped DNA dendrimers, which then captured DNA-peptide conjugates (D13-P), induced lipid molecule assembly to form a Den-siRNA-Lipo structure, and successfully delivered it into the cell, effectively silencing gene expression [80].

Recently, Liu group reported on the study of using FGA strategy to prepare mRNA-LNPs [81]. The study utilized the secondary structure collapse advantage of long mRNA chains themselves, and based on RNA origami technology, the long chain mRNA was folded into compact mRNA nanoparticles using several oligonucleotide short chains. Then, it was used as an amphiphilic framework, using peptides or cholesterol as leading hydrophobic groups (LHGs) to induce phospholipid assembly through hydrophobic interactions (Figure 4E). The article also reported that the mRNA-LNPs prepared by this strategy have high mRNA encapsulation efficiency, good stability, and cell delivery level. It is worth noting that the LNP prepared by this technology can be non-cationic dependent, which have the

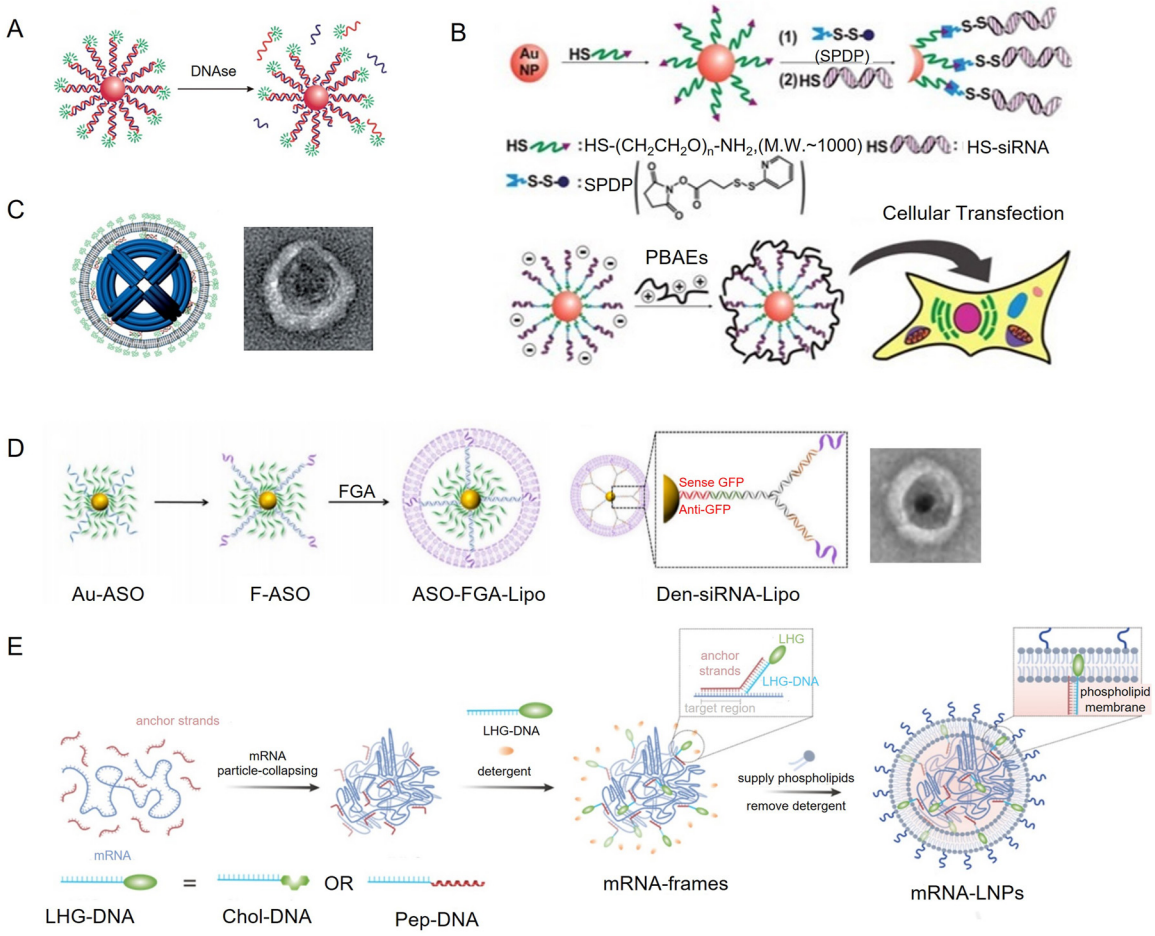

Figure 4: Frame guided assembly (FGA) technology for RNA delivery. (A) Oligonucleotide-modified gold nanoparticles for intracellular gene regulation. Figure reproduced with permission from Ref. [77], Copyright 2022, Springer Nature; (B) Gold, poly (beta-amino ester) nanoparticles bind to siRNA via biodegradable disulfide linkages for siRNA delivery. Figure reproduced with permission from Ref. [78], Copyright 2023, Royal Society of Chemistry: London, UK; (C) A virus-inspired membrane encapsulation of DNA nanostructures similar to FGA strategy. Figure reproduced with permission from Ref. [79], Copyright 2023, Royal Society of Chemistry: London, UK; (D) Preparation and application of high load functional ASO/siRNA delivery carrier based on FGA strategy. Figure reproduced with permission from Ref. [80], Copyright 2023, Royal Society of Chemistry: London, UK; (E) A facile method to prepare non-cationic mRNA-LNP based on FGA strategy. Figure reproduced with permission from Ref. [81], Copyright 2023, Royal Society of Chemistry: London, UK.

capability to avoid the toxicity and immunogenicity of cationic and ionizable lipids at some extent. However, further research is needed on the specific delivery mechanism and *in vivo* therapeutic effects.

In summary, the use of nanoparticles assembled with frame greatly enhances the versatility and efficiency of delivery systems, while also enabling high loading rates. The FGA-LNP technology combines the strengths of LNP technology, origami, and frame nucleic acid technology, making it a promising option for RNA delivery. However, further research is needed to address critical issues such as the stability of the carrier system, potential immunogenicity, therapeutic effectiveness, and potential side effects. Addressing these challenges is crucial for advancing the application of FGA-LNP technology for RNA delivery.

DNA hydrogel

DNA hydrogels are soft, water-swollen three-dimensional structures that are primarily formed through the self-assembly of cross-linked DNA via hybridization, enzyme catalysis, or entanglement [88]. These hydrogels are naturally biocompatible and biodegradable, making them an ideal choice for biomedical applications. Additionally, they offer tunable physiochemical characteristics and injectability, making them highly versatile. DNA hydrogel has received significant attention in drug delivery and has shown promise in RNA delivery. They have the ability to encapsulate and shield RNA molecules from enzymatic degradation, ensuring their stability and delivery efficacy. Furthermore, researchers have been able to engineer DNA

hydrogels to enable controlled release of RNA, further enhancing their potential as RNA delivery systems. Recent advancements in the field have highlighted the potential of DNA hydrogel-based delivery systems for various types of RNA, including small interfering RNA (siRNA), messenger RNA (mRNA), and microRNA (miRNA). These developments hold great promise for the future of RNA-based therapies.

DNA hydrogel as carrier for RNA delivery

DNA hydrogel can be crosslinked by the hybridization or interlocking of branched DNA units through the formation of hydrogen bonds. With DNA bases alone or hybridizing with other materials, they enable the rational design of multifunctional hydrogels [88, 89]. DNA nanosized hydrogel (nanogel) and DNA supramolecular hydrogel both play crucial roles in delivering therapeutic RNAs. DNA nanogels are constructed by using DNA origami, and can exhibit a range of structural and functional properties. Zhang group has made a significant breakthrough in the development of a DNA-grafted polycaprolactone (DNA-*g*-PCL) hydrogel. This hydrogel is formed by using a negative-charged spherical nucleic acid (SNA) structure, along with brushes for hybridizing siRNA or miRNA that are embedded inside the structure to prevent enzymatic degradation. This hybrid nanogel system combines the unique properties of DNA, such as programmability and biocompatibility, with the advantageous characteristics of PCL, as well as offers mechanical strength and degradation properties. The DNA-*g*-PCL brushes were used as the scaffolding for the nanogel, while the siRNA with single-stranded overhangs served as the linker (siRNA-L) to crosslink the DNA-*g*-PCL brushes together (Figure 5A). By tuning the ratio of siRNA-L and DNA-*g*-PCL brushes, the nanogels can be assembled with adjustable size. Additionally, the steric hindrance provided by the extra brushes on the surface of SNA enables nanogel to be protected from RNase digestion, which enhances the stability *in vivo* [7]. The DNA-*g*-PCL-based hydrogel system can therefore expand easily as a general delivery platform, which could be applied to target diverse genes by simply changing siRNA sequences [90, 93, 94]. Apart from siRNAs, Zhang group first reported a novel nucleic acid nanogel-based platform for efficient mRNA delivery. Utilizing the poly(A) tail of the mRNA, they designed nanogels comprising poly(T)$_{20}$-grafted polycaprolactone (T$_{20}$-*g*-PCL) brushes that can easily encapsulate the mRNA through the hybridization between the poly(A) tail of mRNA and DNA linkers (a linear double-stranded DNA with two poly(A)$_{20}$ overhangs). They have successfully demonstrated that the optimized nucleic acid nanogels are capable of encapsulating and delivering mRNAs encoding various proteins, including reporter EGFP and functional nuclease of Cas9, into cells. This nucleic acid nanogel-based platform showcased the potential for delivering mRNAs encoding therapeutic proteins or vaccines (Figure 5B) [90]. In addition to the aforementioned strategies, another valuable approach for transporting designed RNAs is the utilization of functional RNA sequences as the complementary strand or linker to form DNA hydrogel. Komura et al. designed a novel GU-rich RDgel using nanostructured GU-rich RNA/DNA assembly (hexapodRD6) of six phosphordiester DNAs and 20-mer phosphorothioated RNAs. They assembled RDgel with two set of hexapdRD6, which was efficiently taken up by dendritic cells and showed potential as an adjuvant in tumor therapy. This strategy has the ability to sustainably release GU-rich RNA (Toll-like receptor 7 and 8, which induce strong immune responses) in the serum environment, taken up by DC2.4 murine dendritic cells and induced a high level of tumor necrosis factor-α release from these cells when it was incorporated into RDgel [95].

Several types of membranes, including PEG-, PEI-, and erythrocyte-based membrane, were employed to coat nanogel to prevent RNAs from degradation by nucleases, thereby extending their circulation lifetime. Recently, Zhang group successfully developed a virus-mimicking membrane-coated DNA nanogel named Vir-Gel. They utilized DNA-grafted polymer brush to crosslink with miR155. This miR155 loaded DNA gel was further coated with an erythrocyte membrane decorated with two functional peptides, M2pep peptides and HA2 peptide. The M2pep peptide can enhance the delivery of nanogel by specifically targeting M2-microglial and macrophage cells. The HA2 peptide derived from the influenza virus facilities the fusion of erythrocyte membrane and endosomal membrane, leading to increased release of the nanogel within the cells. Once the miR155-bearing nanogel enters the cytoplasm, miR155 is released from the nanogel by RNase H digestion, resulting in increased secretion of pro-inflammatory cytokine and nitrogen synthase, which in turn contributes to reprogram microglia and macrophages from an anti-inflammatory M2-phenotype to a pro-inflammatory M1-phenotype that effectively suppress glioblastoma growth [94].

The delivery efficiency of RNA using DNA hydrogel can be improved by incorporating inorganic materials such as gold nanoparticles, or by introducing modifications such as DNA aptamers and proteins. The assembly of DNA-gold nanoparticle conjugates offers unique properties resulting from the interaction between DNA and the gold nanoparticles. The dense loading of oligonucleotide on the surfaces of the gold nanoparticles acts as the hydrogel core, providing a suitable environment for enhanced stability

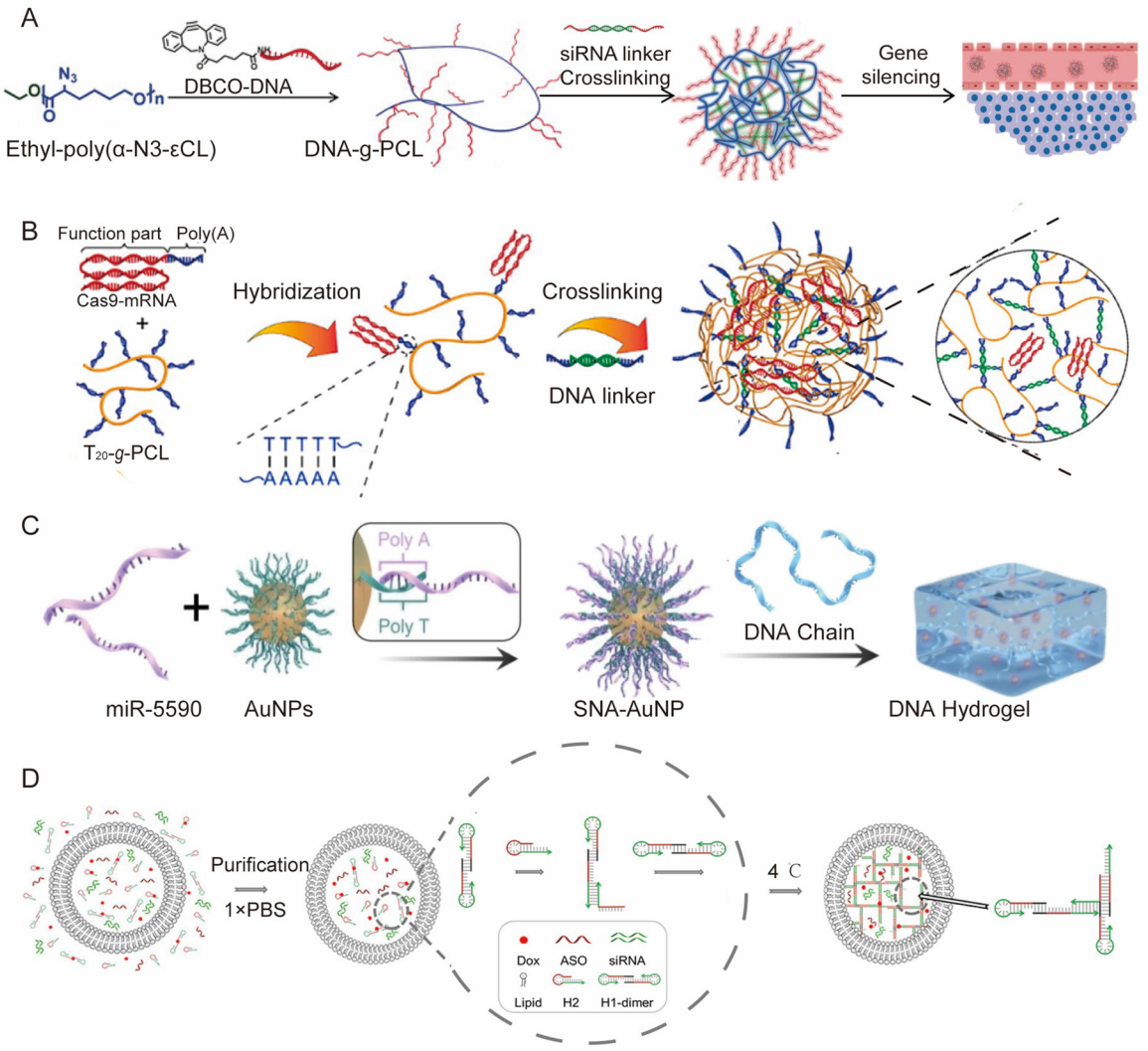

Figure 5: DNA based hydrogel for RNA delivery. (A) A nanosized spherical hydrogel for the delivery of siRNA was constructed using DNA-grafted polycaprolactone (DNA-*g*-PCL) brushes hybridized with siRNAs. Figure reproduced with permission [7], Copyright 2018, Wiley: Hoboken, NJ, USA; (B) Nanogel-based mRNA delivery system featuring poly(T)$_{20}$-grafted polycaprolactone (T_{20}-*g*-PCL) brushes, DNA linker and mRNA with poly-A tail. Figure reproduced with permission from Ref. [90], Copyright 2023, Royal Society of Chemistry: London, UK; (C) A long linear DNA hydrogel loaded with miRNA using AuNP as the spherical nucleic acids. Figure reproduced with permission from Ref. [91], Copyright 2023, Springer Nature; (D) Hydrogel-LNP delivery for siRNA and other therapeutics fabricated with two DNA hairpin precursors. Figure reproduced with permission from Ref. [92], Copyright 2023, American Chemical Society: Danvers, MA, USA.

and the ability to self-assemble into complex structures. For instance, Song et al. loaded miR-5590 via SNA with gold nanoparticles as the core to form a DNA hydrogel (miR-5590-SNA@DNAgel), which facilitate the effective delivery of miRNAs for gene therapy, preventing the leakage of miR-5590-SNA and facilitating the efficient delivery of therapeutic RNAs (Figure 5C) [91]. Similarly, DNA aptamer can also serve as a carrier to deliver therapeutic RNA by encapsulating them in self-healing, injectable long linear RNA/DNA hybridized hydrogel (RDgel). This delivery system offers longer-lasting mechanical strength and provides prolonged and controllable release, leading to improved efficiency in delivering siRNA to target tumors [96]. Zhang Group employed miRNA-34a as crosslinkers to crosslink DNA-grafted polycaprolactone brush (DNA-*g*-PCL) into the nanogel. A complementary short DNA fragment was then conjugated to the cysteine of nanobody (7D12) targeting the epidermal growth factor receptor (EGFR). The resulting Nb-nanogel can selectively target at the tumor site and facilitate cellular internalization. The hybridized miRNA-34a can be cleaved by RNase H, releasing miR-34a, and effectively inhibiting tumor growth [97].

In addition to DNA nanogels, DNA supramolecular hydrogels are self-assembled hydrogel networks that are

formed through the non-covalent interactions of DNA molecules. These supramolecular hydrogels rely on reversible, non-covalent interactions to form and stabilize their gel-like structures, making them appealing for applications that require adaptive and responsive materials. Wei et al. developed a novel approach to enhance the stability of lipid nanoparticles by integrating DNA supramolecular hydrogel with the triggering of two DNA hairpin precursors. This innovative strategy allowed for the stable delivery of both small molecule doxorubicin and nucleic acid drugs such as siRNAs, resulting in efficient induction of apoptosis in lung adenocarcinoma cells. The nebulized lipid nanoparticles carrying siRNA^{IGF1R} demonstrated promising potential for cancer therapy (Figure 5D) [92]. The controllable release of nucleic acid drugs at target sites by DNA-based hydrogel has shown safe and effective delivery of nucleotide acid therapy, leading to significant improvement in immunity activation and tumor suppression. Additionally, the injectable DNA hydrogel has shown potential for tissue repair through the delivery of RNA therapy, highlighting its broader applications in the field of nucleic acid therapeutics.

Stimuli-responsive DNA hydrogel for RNA release

Various types of DNA hydrogels, including both conventional and bio-sensitive ones, have been developed to respond to different stimuli. By incorporating elements that can respond to stimuli, such as pH, miRNA, and enzymes like RNase H and other restriction endonucleases, the structure of the DNA in the hydrogel can be modulated, allowing for targeted release of RNA [94–96, 98, 99]. A recent study has developed a pH-responsive DNA nanogel for the delivery of mRNA into cells. This innovative approach involves the use of rationally designed X-shaped DNA scaffolds and mRNA, which are linked by a pH sensitive C-rich linker at neutral pH to form DNA hydrogel. The researchers successfully delivered Gluc and EGFP mRNA into cells, with the formation of i-motif in lysosome. In this acidic compartment (pH 4.5–5.0), the nanogels dissociated and RNAs are released into cytoplasm to express the encoded proteins (Figure 6A). With the presence of pH sensitive i-motif sequence, the DNA hydrogel shows similar efficiency in delivering Gluc mRNA compared to liposomes, while exhibiting superior protein expression rates [98].

The intercellular cells are equipped with a variety of endonucleases, which have the ability to degrade DNA, RNA, or hybrid DNA/RNA structures, allowing for the controllable biodegradation of DNA hydrogel and subsequent release of its payload *in vivo*. Using well-designed DNA hydrogel structures, the Cas9/sgRNA complex withstand RNase activity, and small RNA molecules such as siRNA or miRNA can be gradually released through the activation of RNase H, which selectively digests DNA/RNA hybridized RNA [7, 94]. Moreover, the lengthy linear DNA chain contained multiple restriction sites can be identified by restriction endonucleases for the purpose of digesting the DNA or DNA/RNA hybridized strand, ensuring the gel to sol transition. The Taq-1 restriction enzyme, which recognizes the DNA/RNA hybridized sequence of TCGA/UCGA, has been employed for the controlled release of RNA through the hybridization of multimeric short hairpin RNAs with functional DNA aptamer (AS1411) and the introduction Taq-1 restriction enzyme site on the DNA/RNA hybridized hydrogel. A siRNA-aptamer complex (SAC) can be released from the digestion of Taq-1 and the complex has the ability to recognize and target the nucleolin (NCL) receptor on the tumor membrane, due to the tumor-targeting DNA aptamer. This makes the hydrogel capable of smart RNA delivery. Furthermore, the researchers utilized another interesting approach to regulate the release speed of siRNA by augmenting the quantity of hydrogel layers and extending the length of the mimicked capillary tube where siRNA loaded hydrogel can be injected to (Figure 6B) [96].

Beyond canonical stimuli, biomolecules competitively bind with crucial components of DNA hydrogel, triggering changes in its physical and biological properties. In this context, the complementary reversible sequence of the colon cancer biomarker miRNA-21, which is secreted by THTC-116 cells, has been strategically integrated into the key sequence of DNA hydrogel. This sequence is designed to release the treatment siRNA upon sensing the specific biomarker, allowing for targeted and intelligent cancer treatment [99]. To achieve logic-controlled siRNA release for spontaneous elimination of target cancer cells in the vicinity, a multicompartmental core–shell DNA hydrogel was fabricated using PEG conjugated with a tandem DNA logical circuit. The DNA sequence circuit contains the individual sensing sequence (D1) that can specifically interact with a cancer biomarker, miRNA-21, to trigger a sequence cascade that can then reacted with D2 to release siRNA. The released siRNA is then efficiently taken up and utilized efficiently by lymphocytes for the elimination of colon cancer cells (Figure 6C) [99]. In conclusion, the various biomolecular present in targeted cellular microenvironment have the potential to be utilized for triggering release of RNA from DNA hydrogel by disrupting the original DNA hydrogel structure. Consideration should be given to the intrinsic structure of the DNA hydrogel in order to control the release rate [88, 99].

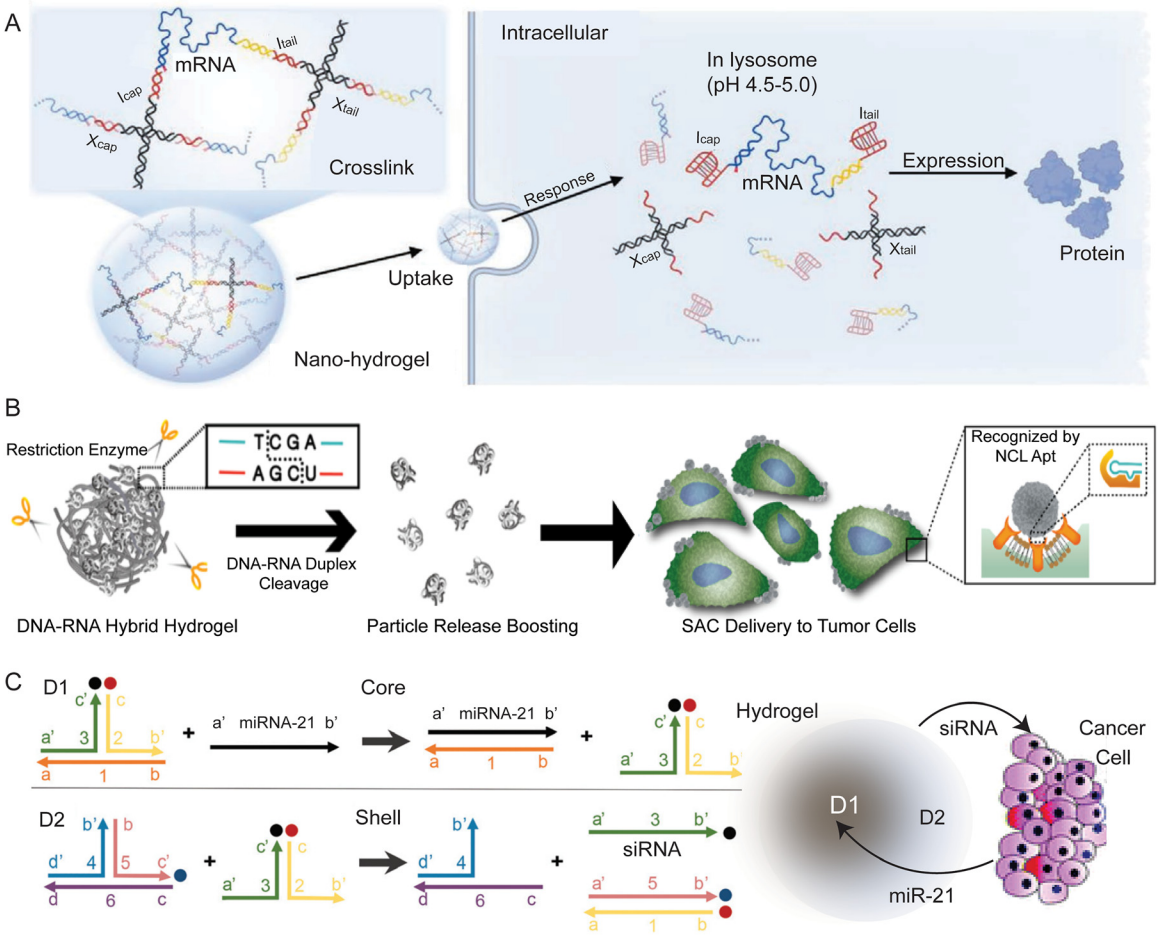

Figure 6: Stimuli responded DNA hydrogel for RNA delivery. (A) mRNA delivery and intracellular pH-responsive release using a DNA nano-hydrogel bearing pH-responsive i-motifs. Figure reproduced with permission from Ref. [98], Copyright 2021, Wiley: Hoboken, NJ, USA; (B) DNA-RNA hybrid hydrogel formed by assembling with DNA templates and siRNA-aptamer complex (SAC). The Taq 1 restriction enzyme induced the release of SACs from the hydrogel. Figure reproduced with permission from Ref. [91], Copyright 2020, American Chemical Society: Danvers, MA, USA; (C) Sensing sequence D1 and treatment sequence D2 are loaded into the core and shell of a hydrogel, respectively. miRNA-21 associated with cancer triggers degradation cascade to release anticancer siRNA. Figure reproduced with permission from Ref. [99], Copyright 2021, Royal Society of Chemistry: London, UK.

Conclusions and future perspectives

The development of RNA delivery and release has emerged as a highly sought-after topic in the field of biomedicine. For instance, in the long-term treatment of chronic diseases, RNA therapy holds the potential to develop long-acting formulations, ensuring consistent drug delivery while reducing the frequency of administration and alleviating patient treatment pain. The current global focus on researching and developing RNA delivery underscores the pressing need for safer, more effective, and advanced delivery systems for small nucleic acid drugs or mRNA vaccines. This review primarily delves into the architecture and construction of DNA-based nanostructures including DNA hydrogel, DNA origami, and FGA-LNP in the context of RNA delivery, as well as their biomedical applications.

Advances in nucleic acid fabrication have made it increasingly cost-effective to obtain RNA materials, providing a strong foundation for research and development of more effective delivery systems. Nonetheless, the development of excellent RNA delivery carriers still faces numerous challenges. For example, delivery carriers need to overcome physical (lipid bilayers) and biological barriers (serum nucleases and scavenge macrophages etc.) to achieve ideal delivery efficiency, and the application of drug carriers in organisms requires strict sample purity to avoid unknown and uncontrollable side effects caused by unstable doses. As a delivery carrier, different carrier components and delivery characteristics can result in various targeted specificity and immunogenicity, which also need to be considered in

practical applications. DNA origami technology possesses the properties of high designability, precise addressability, and biological affinity. In the research and application of delivering RNA drugs, researchers designed DNA origami structures that load RNA molecules by utilizing complementary RNA and DNA strands to form DNA-RNA hybrid chains. RNA molecules of different lengths can be delivered through DNA origami structures of different shapes. By loading siRNA into the origami structure of octahedral or soccer-ball shaped framework DNA, a certain degree of shielding effect can be achieved, which hinders RNase enzyme degradation and protein binding siRNA through space during transportation [47, 51]. By modifying the structure, targeted delivery and controlled release of molecules can be achieved [41]. Biomimetic structural design can also improve delivery advantages [51]. Although it has been proven to be low toxicity in a large number of reports, it is necessary to conduct a comprehensive evaluation of the DNA origami structure before implementing them in clinical practice, observe and evaluate the effects, and the complexity of the DNA origami structure may still lead to inevitable difficulties, especially the large number of DNA single strands used in the evaluation of clinical drugs, which greatly increases the difficulty of evaluation.

It is worth mentioning that the ideal origami structure can also be assembled using mRNA as a scaffold. Andersen et al. reported the design of an octahedral RNA origami structure using the unstructured part of the target mRNA sequence as the scaffold chain, while embedding intrinsic functional siRNAs. By incorporating recognition sites for Dicer at selected locations in the structure, the production of functional siRNAs was promoted [100]. Ding group designed an RNA-DNA hybrid origami composed of mRNA scaffolds, where the DNA-RNA hybrid switch chain site will be recognized and cleaved by RNase H enzyme in the organism, and releases the antisense single strand [101]. However, this hybrid chain is not as stable as RNA-RNA and DNA-DNA hybridization. In particular, Hu et al. utilized flexible RNA origami technology to directly fold Smad4 mRNA into a "nano lantern" structure through two customized RGD (Arg-Gly-Asp) modified circular RNA nail staples to fold long mRNA molecules, achieving successful cell delivery and expression of mRNA without the need for a large number of ssDNA [6]. However, there are currently no reports directly using DNA origami for mRNA delivery, possibly due to the difficulty of DNA origami-RNA conjugation caused by the complex secondary structure of mRNA itself. And the RNA strand itself is easily degraded by nucleases in the environment, making it difficult to control how to maintain the stability of the mRNA strand during the subsequent processing of being encapsulated in the DNA origami structure. The implementation of viral capsid coatings has proven to protect encapsulated DNA or RNA-DNA origami structures from degradation, effectively enhancing the cell delivery efficiency of DNA nanostructures [102]. Research has demonstrated a remarkable 13-fold increase in the cell delivery efficiency of capsid protein modified DNA origami complex compared to unmodified origami structures [103]. Additionally, these modified DNA origami octahedra without envelope showed a slower *in vivo* degradation rate [79]. The successful application of these modifications may serve as a valuable reference for future RNA delivery methods. The advancement of RNA delivery via DNA origami still requires substantial efforts. There is a need to improve structural design maintaining the simplicity and efficiency of the DNA structural components delivered to the organism. Additionally, efforts are required to streamline production processes and reduce manufacturing costs. Effective *in vivo* RNAs release while maintaining stable delivery, as well as controlling RNA storage and transportation conditions to preserve structural integrity, are also key areas for improvement. Solutions to these challenges are likely to emerge as DNA nanotechnology continues to evolve.

Majority of current RNA-delivery are based on research involving cationic liposomes and ionizable liposomes. The assembly principles for these technologies are relatively simplistic, relying on charge adsorption. The FGA-LNP technology is expected to avoid the dependence of cationic liposomes or ionizable liposomes by providing a determinate structural morphology and size with the frame core, making it highly designable through modification of DNA strands. Overall, these advantages suggest that FGA-LNP technology has significant potential for RNA delivery applications. However, its research and application are still in the early stages of development, and there are still many problems that need to be addressed, such as the compatibility of carrier delivery for FGA-LNP preparation and the production of a large number of structures.

In addition to aforementioned strategies, there are numerous innovative approaches being developed for RNA delivery. One such example is the construction of an LPP (lipopolyplex) nano-delivery platform, which consists of a bilayer structure comprising a poly-(β-amino ester) polymer loaded with mRNA as the core and phospholipid wrapped as the outer shell [104]. This virus-like nanoparticle with a core–shell structure has shown effective delivery of mRNA. Furthermore, Li et al. demonstrated successful cellular delivery and therapy using a nanoframework carrying siRNA, which was constructed through precipitation polymerization with acrylate-DNA as a cross-linking agent [105]. It is worth noting that the

exploration of RNA delivery carried by polymer nanoframe has shown great potential and has attracted increasing attentions [106].

Recent studies have demonstrated a remarkable advancement in the therapeutic potential of RNA drugs administered through DNA hydrogels. These findings have revealed a substantial enhancement in therapeutic efficacy, particularly when these RNA drugs are utilized in conjunction with other therapeutic modalities. However, it is still valuable to pursue the development of pure nucleic acid hydrogels in order to minimize the negative impact of impurities that are generated during fabrication. To achieve stimuli RNA release, various elements have been employed into RNA deliver carriers. Utilizing dual or multiple stimuli-responsive DNA hydrogel can lead to accurate release at specific target sites, thereby enhancing efficiency and targetability. However, ensuring that each functional component responds appropriately to the intended stimuli collaboratively without interfering with each other's responsiveness is a complex task. Further research is necessary to gain a deeper understanding of how DNA hydrogels react to external stimuli and to further advance their application in controlled RNA release. It is theorized that DNA hydrogels responding to diverse stimuli may exhibit distinct kinetic characteristics. However, there is a paucity of theoretical kinetic studies on DNA hydrogels. More in-depth investigation into the kinetics of these hydrogels is warranted. Additionally, although gene editing-associated RNAs (such as Cas9-mRNA, sgRNA or the Cas9/sgRNA complex) have been delivered and released in cell via DNA hydrogel, the knock down efficiency of EGFP remains low and there have been no reports of gene editing for cancer as of yet [90, 107].

Progress in clinical trials for DNA nanostructure-mediated RNA delivery, including DNA hydrogel, DNA origami, and FGA-LNP technology, continues to face formidable challenges. One major obstacle is the lack of comprehensive reporting on the safety and long-term efficacy of the different components of DNA structures in drug evaluation. Additionally, off-target binding and the efficient delivery of RNA-based drugs to specific organs and tissues present significant hurdles. Furthermore, the potential for sequence-induced toxicity remains a critical consideration for the future implementation of these delivery technologies. Overcoming these challenges is crucial for the future clinical application of DNA nanostructure-mediated RNA delivery. The development of precise, high-purity long-strand synthesis can offer valuable assistance in addressing these issues. Moreover, advanced artificial intelligence (AI) and machine learning (ML) techniques can be utilized to design and simulate hydrogel behavior under various conditions, enabling the customization of properties and enhancing the specificity and safety of RNA delivery method. As these challenges are gradually addressed, DNA nanostructure-mediated RNA delivery holds great promise for revolutionizing the field of RNA delivery and personalized RNA therapy.

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