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# Therapeutic DNAzymes: From Structure Design to Clinical Applications

Jiaqi Yan, Meixin Ran, Xian Shen,\* and Hongbo Zhang\*

Therapeutic DNAzymes have unceasingly intrigued the scientific community owing to their prosperous gene regulation capability. The efficacy of DNAzymes against many types of diseases has been extensively studied for over two decades. However, the high expectations for DNAzymes are still not translated to the clinic because of their low effectiveness *in vivo*. Over the last five years, several aspects have been considered to optimize DNAzyme-integrated therapeutics, including structural stability, mechanism exploration, cell internalization rate, cofactor activation, and off-target effects. Hence, this review first discusses the early monotherapy and structural design of DNAzymes. Subsequently, the latest modes of action are reviewed, followed by an elaboration on structural stabilization strategies considering the catalytic core and substrate-binding arms. DNAzyme-based synergistic therapy is then examined, highlighting responsive carrier construction, synergistic effects, the latest discovered advanced functions, and off-target concerns. Beyond this, key clinical advances in DNAzyme-based therapy are elucidated by showcasing clinical progress. Finally, future trends and development challenges for DNAzyme-powered therapeutics in the coming years are discussed in detail.

## 1. Introduction

DNAzymes were first discovered by Breaker and Joyce in 1994.<sup>[1]</sup> Their creation marked a great breakthrough in therapeutics and biocatalysis. To date, no DNA sequence with catalytic function has been found occurring naturally. These man-made enzymes have been actively used in both biosensing and therapeutics for the past 29 years.<sup>[2]</sup> In the biosensing field, in addition to their well-known metal-sensing activity,<sup>[3]</sup> DNAzymes also respond to bacterial lysate components,<sup>[4]</sup> biotargets, such as DNA,<sup>[5]</sup> microRNA (miRNA),<sup>[6]</sup> and specific gene sequences,<sup>[7]</sup> and adenosine triphosphate (ATP).<sup>[8]</sup> The field of biosensing has consistently grown since 2000,<sup>[3]</sup> with numerous outstanding summarizing and predicting reviews.<sup>[9]</sup>

However, from the perspective of development, the DNAzyme expansion process in therapeutic areas is vastly different from that in biosensing. Showing promise, DNAzymes as therapeutics have been

broadly studied *in vivo* since 1999.<sup>[10]</sup> As gene-regulatory agents, RNA-cleaving DNAzymes can recognize and silence specific gene sequences.<sup>[11]</sup> In addition, DNAzymes possess tremendous merits, such as high selectivity,<sup>[12]</sup> multiple turnover rates, no permanent influence on the genome, simple design, and suitability for large-scale rapid production, which have great clinical application value.<sup>[13]</sup> Through considerable exploration over the past two decades, DNAzyme-related therapeutics have covered cancer, viral, asthma, cardiovascular, inflammatory, and central nervous system diseases.<sup>[14]</sup> Nevertheless, more in-depth research on DNAzymes revealed that *in vivo* treatment is limited by several obstacles. For example, indispensable metal cofactors can be seen as an advantage for metal-ion monitoring, but the lack of metal ions within physiological conditions limits the target RNA depletion ability of DNAzymes.<sup>[15]</sup> Hence, the expansion of therapeutic DNAzymes for treatment *in vivo* has gradually shifted from its early flourishing, manifesting as sluggish clinical studies.


In the last five years, rapid advancements in nanomedicine and synergistic concept maturation have brought a second stage of promise for therapeutic research on DNAzymes. The logical relationships behind DNAzyme-based therapeutics and biosensing are closely blended. For instance, the concept of building biomarker sensing systems can be employed when designing specific RNA-cleavage DNAzymes with biomarker target

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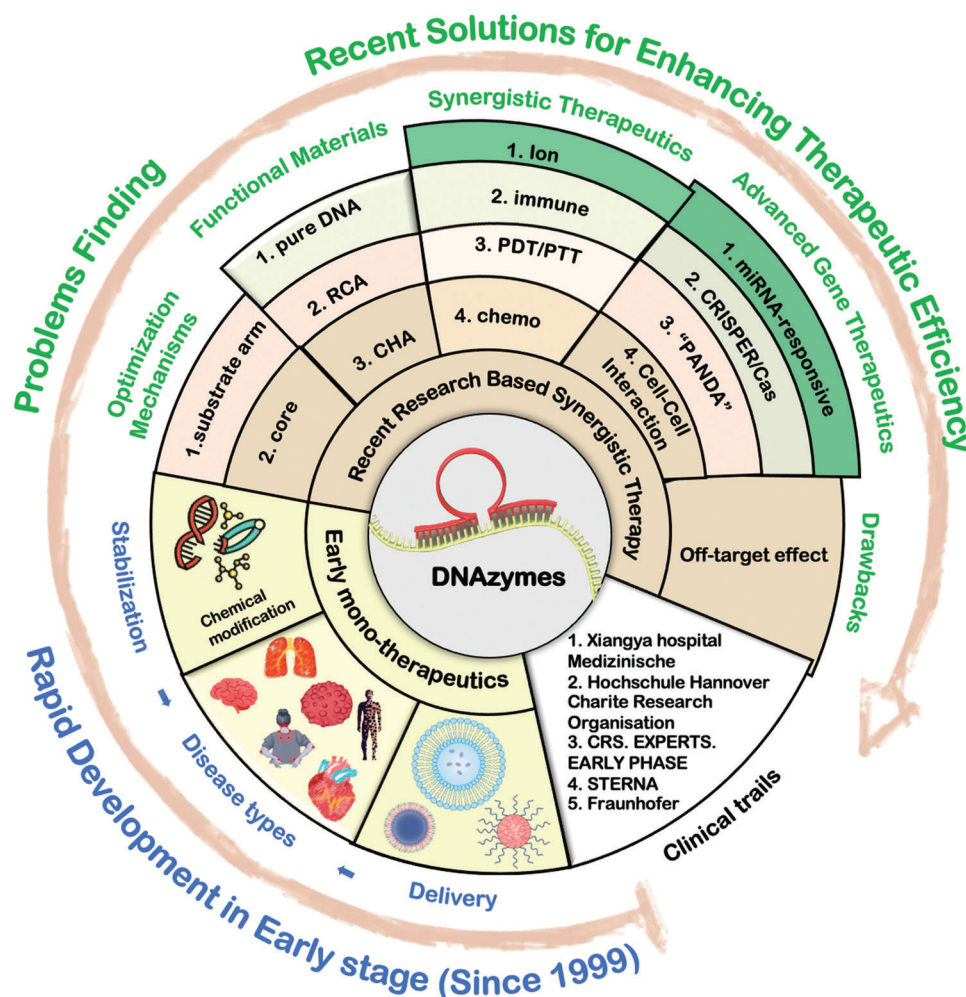
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**Scheme 1.** Schematic of DNAzyme-based therapeutic research, from early studies to the latest investigations (RCA rolling circle amplification, CHA catalytic hairpin assembly, PTT photothermal therapy, PDT photodynamic therapy).

therapeutic effects.<sup>[16,17]</sup> In addition, sensing based on photoreponsive DNAzymes<sup>[18]</sup> can also be applied in light-triggered DNAzyme-integrated synergistic therapy. This promising stage is also reflected in the extensive combination of DNAzymes with other therapeutics and an increasing number of publications. However, systematic reviews summarizing and analyzing therapeutic DNAzyme research ideas and directions are scarce, especially over the past five years.

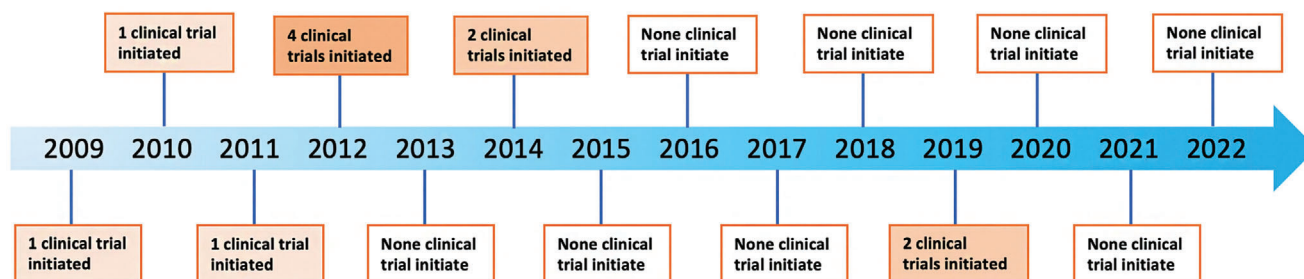
In this review, we dissect therapeutic DNAzyme evolution in time, as well as the perspectives of DNA functional materials<sup>[19]</sup> (Scheme 1). We delineate the entire development process, from early structural modifications, disease treatment directions, and drug delivery strategies, to the latest exploration of catalytic mechanisms, combination treatment strategies, and state-of-the-art novel functions. Through backtracking and cutting-edge follow-up, we clearly show the expansion of therapeutic DNAzymes to obtain experience from early research failure and gain new understanding and application ideas for current technology. Moreover, we also regard DNAzymes as a building block for the construction of biological functional materials, to deeply analyze their role as the basic unit of material design. In addition, the

current clinical situation and future research directions are described.

## 2. Background of DNAzyme-Based Therapy

During its rapid expansion since 1999, DNAzyme-based treatment has been employed in vitro/vivo in clinical studies, and its safety and effectiveness have been verified in humans. Owing to their easy synthesis and design flexibility, DNAzymes have been widely tailored and used in an extremely wide range of studies, including antiviral,<sup>[20]</sup> anti-inflammatory,<sup>[21]</sup> anticancer,<sup>[22]</sup> and the treatment of cardiovascular<sup>[23]</sup> and central nervous system diseases.<sup>[24]</sup> Despite the lack of novel delivery systems and synergistic effect in combination with other advanced strategies, this period of advancements provided a solid foundation for the recent design and therapeutic applications of DNAzymes.<sup>[25]</sup>

However, the current clinical enthusiasm for DNAzymes is much lower than that between 2009 and 2014. This is due to their low activity in vivo, which has led to stagnating clinical research (Figure 1). Specifically, stability modification of DNAzymes lacks theoretical guidance. Without deep analysis of conformation and



**Figure 1.** Number of clinical programs launched per year.

action mechanism of DNAzyme/RNA complex,<sup>[13b]</sup> the modification processes rely heavily on large-scale screening. Thus, the catalytic performance and stability of DNAzymes could not be enhanced simultaneously. Moreover, the concentration of metal cofactors in diseased tissues is insufficient, resulting in low catalytic efficiency. More importantly, although DNAzymes can recognize specific genes, they still induce off-target effects and cannot silence genes in specific diseased tissues.

Hence, in this section, we first summarized the rapid development period of DNAzymes, which highlights the DNAzyme-based monotherapeutic system, including different disease treatments, as well as drug delivery systems. Later, we emphasized the problems identified during this vigorous development period, including contradictions and conflicts in structural modification and the issue of insufficient coenzymes.

## 2.1. A Stage of Rapid Development

### 2.1.1. DNAzyme-Based Monotreatment for Different Diseases

DNAzyme-based therapies cover a wide range of diseases, including viruses, inflammation, cancer, cardiovascular, and central nervous system diseases (Table 1). Among the different therapeutic DNAzymes, 10-23 DNAzyme played a leading role in early treatment. In general, DNAzyme-based therapy initially tended to focus on the ability to silence specific messenger ribonucleic acid (mRNAs), and the adenine-uracil (AU) nucleotide within the “start codon” of RNA was usually set at the 10-23 DNAzyme cleavage position. DNAzymes can be designed as regulators for different gene sequences according to the purpose of disease treatment.

**Table 1.** DNAzymes as early stage therapeutic agents for different diseases..

Disease	Target	Outcome	Ref.
Viral diseases	HIV	Inhibit viral replication	[26]
	Hepatitis B virus	Suppress the corresponding antigen proteins	[28]
	Human rhinovirus	Inhibit transcription and translation	[15a]
Antiangiogenic	EBV	Impact tumor microvascular permeability	[29]
	VEGF-A	Reduce neovascularization	[33]
	VEGF-R2	Inhibit tumor angiogenesis	[36]
	IGF-II	Inhibit the invasion and migration of liver cancer cells	[39]
Antimetastatic	12-LOX	Inhibit tumor vascular proliferation	[40]
	<i>Akt1</i>	Strongly inhibit <i>Akt1</i> mRNA and protein expression in the NPC cell line CNE1-LMP1	[43]
	EBV LMP1	Inhibit cancer proliferation and enhance radiosensitivity	[44]
Cardiovascular disease	MMP-9 mRNA	Inhibit both angiogenesis and metastasis	[46]
	PAI-1 mRNA	Realize regeneration and functional heart recovery	[47]
	TNF- $\alpha$	A significant reduction in left ventricular end diastolic pressure and lung weight, accompanied by increases in arterial blood pressure and myocardial blood flow	[49]
Anti-inflammation therapy	GATA3	Significantly attenuate early or even late asthma responses triggered by allergens in patients with allergic asthma	[52]
	<i>iNOS</i> gene	A significant decrease in NO levels in the serum and peritoneal lavage	[21]
	TGF- $\beta$	Useful in glomerulonephritis treatment	[54]
Central nervous system diseases	Twist	Decrease transcription	[24,55]

First, DNazymes are conducive for the treatment of viral diseases. Viruses often have very simple elements, with the most basic components including genetic material (DNA and RNA) and proteins, and sometimes modifications of sugar and lipid units. The genetic material of the virus can be either DNA or RNA, which is also divided into one or two strands. Whether DNA or RNA, double- or single-stranded, these genetic materials play a decisive role in guiding the synthesis of viral proteins. Hence, DNazymes can serve as viral mRNA cleavage machines to mediate the downregulation of viral proteins. For example, human immunodeficiency virus-1 (HIV-1) integrase is a protein with a molecular weight of 32 kDa, encoded by the HIV *pol* gene, and an essential enzyme for HIV replication. In this case, 10-23 DNzyme was utilized to inhibit HIV-1 integrase gene expression,<sup>[26]</sup> and viral replication was significantly inhibited. By contrast, HIV fusion and internalization can be enhanced by binding C-C chemokine receptor type 5 (CCR-5) and C-X-C chemokine receptor type 4 (CXCR-4) receptors on host cells. Therefore, DNazymes have also been used to silence host cell receptor mRNA to reduce viral internalization.<sup>[27]</sup> Furthermore, other types of viruses have been treated using DNazymes, including hepatitis B virus,<sup>[28]</sup> human rhinovirus,<sup>[15a]</sup> and Epstein-Barr virus (EBV),<sup>[29]</sup> suggesting that DNazymes are an effective means to treat viruses. In addition to antiviral treatment, DNazymes can also be used to detect viruses, including severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).<sup>[30]</sup>

DNazymes can be used against cancer from several perspectives, including antiangiogenic, antimetastatic, tumor cell apoptosis induction, and cancer-related kinase inhibition. Early treatment based on DNazymes mostly involved silencing oncogenes and focused on a single treatment.<sup>[31]</sup> Wu et al. first used DNazymes for cancer treatment<sup>[32]</sup> to deplete the *bcr-abl* gene for cancer cell suppression. Detecting fresh CD34<sup>+</sup> (CD = cluster of differentiation) bone-marrow cells from patients with chronic myeloid leukemia showed that DNazymes could specifically inhibit 80% of the growth of *bcr-abl*-positive colonies, which confirmed their therapeutic ability.

DNazymes can be used as antiangiogenic agents. Vascular endothelial growth factor A (VEGF-A) plays an important role in angiogenesis, vascular permeability, and metastasis during tumorigenesis. It has been demonstrated that early growth response (EGR)-1 induced by the extracellular-signal-regulated kinase pathway can activate VEGF-A in lung cancer cells.<sup>[33]</sup> Hence, an investigation found that intratumorally delivering EGR-1-targeted DNazymes could effectively reduce neovascularization.<sup>[34]</sup> VEGF receptor 2 (VEGF-R2) is another potential target to inhibit tumor angiogenesis.<sup>[35]</sup> DNazymes can achieve tumor angiogenesis inhibition by cleaving VEGF-R2 mRNA.<sup>[36]</sup> Zhang et al. found that DNazymes could effectively digest the VEGF-R2 mRNA substrate in a concentration- and time-dependent manner. In addition to revealing antiangiogenesis function through *in vitro* experiments, a DNzyme complex within a nonviral vector could also significantly inhibit tumor growth *in vivo*. After the fourth injection, compared to the control, the tumor size in the DNzyme treatment group decreased by nearly 75%.<sup>[36]</sup> Another example is insulin-like growth factor II (IGF-II), a mitogenic peptide overexpressed in liver cancer cells.<sup>[37]</sup> IGF-II directly promotes angiogenesis in liver cancer.<sup>[38]</sup> Thus, DNazymes with IGF-II-silencing effects

were utilized to inhibit the invasion and migration of liver cancer cells.<sup>[39]</sup> VEGF and matrix metalloproteinase (MMP) were also downregulated in DNzyme-treated cells, which verified the function of DNazymes as a new therapeutic molecule with a liver-cancer-cell-targeting effect.<sup>[39]</sup> Notably, human-platelet-type 12-lipoxygenase (12-LOX) and MMP9 play key roles in promoting angiogenesis in prostate cancer,<sup>[40]</sup> and 12-LOX inhibition using DNazymes as gene regulation agents can inhibit the vascular proliferation of tumors.<sup>[41]</sup> In summary, tumor angiogenesis is mediated by multiple mechanisms. Different matched DNazymes can be developed to cope with this phenomenon by silencing tumor-angiogenesis-promoting genes.

Antimetastasis is another main topic in DNzyme-based cancer therapy. The protein kinase *Akt1* is related to tumor progression and metastatic signaling pathways and has been considered as a promising target for gene silencing.<sup>[42]</sup> Cao and co-workers utilized DNazymes to intercept *Akt* for nasopharyngeal carcinoma (NPC). DNzyme strongly inhibited *Akt1* mRNA and latent membrane protein 1 (LMP1) expression in the NPC nasopharyngeal carcinoma epithelioid cell line (CNE1). Further studies have also shown that DNazymes can inhibit cell proliferation, stimulate apoptosis, and inhibit the growth of xenografts in nude mice.<sup>[43]</sup> Another similar example is the EBV LMP1, which regulates the reaction of stromal cells in the tumor microenvironment to promote cancer progression and metastasis. DNazymes have been applied to downregulate LMP1 expression in cancer cells, which inhibits cancer proliferation and enhances radiosensitivity.<sup>[44]</sup> Moreover, the abovementioned MMPs were also correlated with both angiogenesis and metastasis. Yang et al.<sup>[45]</sup> and Hallett et al.<sup>[46]</sup> used DNazymes to silence MMP-9 mRNA in different cancer cell lines, exhibiting great prospects.

DNazymes can be used to treat cardiovascular disease.<sup>[23]</sup> After DNazymes with specific plasminogen activator inhibitor-1 (PAI-1) mRNA cleavage function were injected into the area around the infarction in rats, PAI-1 expression significantly declined. This improved the neovascularization of the infarcted tissue. In addition, inhibition of PAI-1 gene expression using DNazymes has enabled regeneration and functional heart recovery.<sup>[47]</sup> Another example is activation of the tumour necrosis factor alpha (TNF- $\alpha$ ) signal transduction pathway, which may lead to vascular dysfunction and the occurrence and development of atherosclerosis. In severe cases, myocardial infarction and heart failure may be induced.<sup>[48]</sup> The delivery of TNF- $\alpha$ -targeted DNazymes in rats with heart failure can mediate a significant reduction in left ventricular end-diastolic pressure and lung weight, accompanied by an increase in arterial blood pressure and myocardial blood flow.<sup>[49]</sup>

DNazymes are good candidates for anti-inflammatory therapies. Bronchial asthma is a chronic inflammatory disease. DNazymes can be used to treat asthma by downregulating *GATA3*.<sup>[50]</sup> Krug et al.<sup>[51]</sup> reported that the use of SB010 (DNzyme targeting *GATA3* mRNA) significantly attenuated early or even late asthma responses triggered by allergens in patients with allergic asthma. Caramori et al. also claimed that *GATA3*-specific DNazymes can be used to explore the pathophysiological effects of *GATA3* expressed by pulmonary resident and inflammatory cells in asthma.<sup>[52]</sup> Other anti-inflammatory conditions can also be treated through DNzyme-powered gene silencing. For instance, increased NO production by nitric oxide

synthase (*i*NOS) also mediates various inflammatory diseases.<sup>[53]</sup> DNAzyme treatment resulted in a significant decrease in NO levels in serum and peritoneal lavage, which confirmed the functional inhibition of the *i*NOS gene in lipopolysaccharides (LPS)-injected mice. In addition, DNAzymes can limit the excessive NO produced via costimulation by cytokines and LPS in mouse peritoneal macrophages.<sup>[21]</sup> Another study strongly suggested that inhibiting transforming growth factor beta (TGF- $\beta$ ) expression in the mesangium is an important treatment strategy to prevent the progression of renal fibrosis. Therefore, glomerulonephritis can be cured using deoxyribonuclease (DNase), which inhibits TGF- $\beta$ . Hori and co-workers developed a new gene transfer system using electroporation *in vivo*. DNAzyme solutions were injected into the renal artery, and then an electric pulse was stimulated using tweezer-type electrodes, which can introduce genes into mesangial cells in almost all glomeruli. These results indicated that inhibiting TGF- $\beta$ 1 expression via electroporation-mediated DNAzyme transfer might be useful to treat glomerulonephritis.<sup>[54]</sup>

DNAzymes also have important functions in other central nervous system (CNS) diseases, providing powerful support for gene therapy.<sup>[24,55]</sup> For example, Twist is a basic helix–loop–helix transcription factor involved in the regulation of cell differentiation and apoptosis. Studies have shown that it plays a role in skull development and leads to skull fusion. Phylactou and co-workers utilized DNAzymes as downregulators of Twist expression. Full-length Twist mRNA cleavage results showed that the Twist mRNA level decreased significantly after DNAzyme transfection into C3H10 cells. This was accompanied by a significant increase in p21 mRNA level. Finally, DNAzyme transfection led to an increase in apoptosis, showing the importance of Twist in the apoptosis pathway.<sup>[56]</sup>

In this section, we described the therapeutic strategies achieved using DNAzymes as early monotherapy. Although this development process did not emphasize on cooperative treatment strategies, as well as combinations with modern tissue engineering materials and metal coenzymes, these experiments demonstrated the effectiveness of DNAzymes, forming a brilliant chapter in their historical development.

### 2.1.2. Delivery Systems for DNAzyme-Based Monotherapy

Nanobiotechnology can help DNAzymes achieve efficient catalytic activity in target tissues.<sup>[57]</sup> Nonviral transfection vectors are generally employed to deliver oligonucleotide-based systems, owing to their safety and stability. Before 2017, DNAzyme delivery using nonviral carriers mostly used nanomaterials, such as liposomes, polymers, and metal-based nanoparticles.<sup>[58]</sup>

Liposomes are composed of phospholipids with a single or bilayer structure. Their hydrophilic inner and hydrophobic outer layers can be loaded with drugs with different physicochemical properties.<sup>[59]</sup> Liposomes can be prepared as particles of different sizes, which are biodegradable and nontoxic. In addition, by modifying the liposome surface, targeted drug delivery can be achieved at specific physiological sites. Hence, the liposome system is a good option for DNAzyme delivery, as it can prolong retention time *in vivo*. For instance, vascular smooth muscle cell (SMC) proliferation is key to failure of vein

transplantation after coronary artery bypass grafting. C-Jun plays an important role in SMC proliferation and intimal hyperplasia. Li et al.<sup>[60]</sup> used cationic liposome preparations to deliver DNAzymes targeting the transcription factor c-Jun, which were safe and well-tolerated after local administration. Compared with nonfunctional DNAzymes with catalytic domain mutations, the DNAzyme system in nanocarriers significantly reduced SMC proliferation and c-Jun protein expression *in vitro*. Commercial liposomes, such as Lipofectamine 2000, are also a good choice for DNAzyme delivery. EBV-encoded LMP1 mediates the occurrence and development of NPC. Ke et al.<sup>[61]</sup> used Lipofectamine 2000 as a DNAzyme transfection reagent and found that it effectively inhibited LMP1 expression and reduced the cell proliferation rate. DNAzymes targeting LMP1 effectively controlled tumor growth *in vivo*; therefore, they may have therapeutic significance for NPC treatment.

Polymers are considered ideal drug delivery materials because of their biodegradability, biocompatibility, water solubility, storage stability, and other physical and chemical properties.<sup>[62]</sup> Depending on their structure, polymers can be divided into linear polymers, dendrimers, and hyperbranched polymers. When used as drug delivery systems, polymers with different structures can carry drugs in various ways according to their respective advantages, including encapsulating drugs in reservoirs with polymer coatings (reservoir type), embedding drugs in a polymer matrix (integral type), carrying drugs via polymer–drug conjugation, and introducing targeting factors to deliver drugs to specific disease sites.<sup>[63]</sup> Therefore, DNAzymes combined with polymers for targeted delivery show promising prospects. Aurora kinase A overexpression is highly associated with the malignant phenotype of prostate cancer. Xing et al.<sup>[64]</sup> achieved targeted DNAzyme delivery targeting aurora kinase A using the *N*-acetyl-L-leucine-polyvinyl imine polymer derivative as a drug carrier. Flow cytometry and confocal laser scanning microscopy showed that the polymer could achieve cellular uptake of nanoparticles through energy-dependent and reticulin-mediated pathways, and a higher DNAzyme concentration in the cytoplasm could be obtained through further endosome escape.

Compared with organic nanoparticles, inorganic materials have the advantages of controllable characteristics, such as uniform size, large surface area, and easy surface functionalization.<sup>[65]</sup> In addition, inorganic nanoparticles have unique photothermal and electromagnetic properties, which can endow them with potential functions, such as synergistic therapy and targeted tracing imaging.<sup>[66]</sup> Therefore, the application of inorganic materials, such as gold nanoparticles (AuNPs) and silica carbon materials, in nucleic acid drug delivery systems has received extensive attention. Combining inorganic nanomaterials with DNAzymes can significantly improve the efficiency of gene knockout. Yeh et al.<sup>[67]</sup> combined DNAzyme on the AuNP surface and used it to downregulate the expression of the *GDF15* gene, which is highly expressed in breast cancer. Studies have shown that when DNAzyme binding to the AuNP surface can increase its resistance to nucleases, which is expected to solve the key limitations of *in vivo* research. Subsequently, the same research group achieved TNF- $\alpha$  silencing using this design.<sup>[68]</sup> In comparison, the authors found that AuNPs had a higher transfection efficiency than Lipofectamine. Hepatitis C virus (HCV) is a single-stranded RNA virus. A potential therapeutic

**Table 2.** Different DNAzyme modifications..

Type	Advantages	Disadvantages	Modification	Ref.
3'-inversed dT	Resist enzymatic degradation primarily from 3'-exonucleases, safe and stable, increased catalytic rate	Slower product release rate	Right or reversed 3'-thymidine orientation Recognition arm DNAzymes (7 + 7, 9 + 9, 8 + 8 bp)	[71, 72] [10, 73]
Phosphorothiate linkages	Protect oligonucleotide sequences from degradation, enhance cleavage efficiency	Antisense oligodeoxynucleotides are toxic, immunoreactive, and increase the affinity for cellular proteins, producing sequence-independent reactions	–	[75, 80–82]
2'-O-methylation	High biostability, increased oligonucleotide binding affinity	–	Four or five 2'-O-methyl monomers at the 5' and 3' ends of the substrate recognition arm	[15a, 76]
LNAs	Inhibit mature and precursor miRNAs, raise silencing efficiency, increase oligonucleotide binding affinity	Affect catalytic activity and biological potency	Two or four modified LNA monomers	[77, 83]

strategy involves silencing the HCV genome, thus preventing the virus from replicating in host cells. Ryoo et al.<sup>[69]</sup> constructed iron oxide nanoparticles modified with a cell-penetrating peptide to deliver DNAzymes targeting the HCV gene *NS3*, which encodes helicase and protease. The hybrid system had a strong ability to knockout *NS3* mRNA without adverse reactions.

Another interesting example in 2015 was the use of DNAzyme cofactors as building materials for nanocarrier construction. The 10-23 DNAzyme requires at least 5 mM Mg<sup>2+</sup> ions for efficient catalysis. However, in vivo, it only contains less than 2 mM Mg<sup>2+</sup>,<sup>[70a]</sup> which is less efficient. Fan et al.<sup>[70b]</sup> used MnO<sub>2</sub> as a DNAzyme carrier to deliver the coenzyme factor Mn<sup>2+</sup>. The DNAzyme–MnO<sub>2</sub> system inhibited the expression of *EGR-1* mRNA by 60%. However, the authors did not mention the therapeutic effect of the Mn<sup>2+</sup> cofactor.

In summary, early DNAzyme delivery systems were singular and relied on mimicking conventional oligonucleotide-based drug carriers. Nevertheless, DNAzymes have unique chemical properties compared to those of other nuclear-acid-based agents. They can mediate the degradation of RNase-H-based target sequences through traditional complementary base pairing and employ special nucleic acid catalytic cleavage mechanisms. In addition, DNAzymes require the assistance of a metal cofactor. Therefore, the delivery vector should be tailored according to each unique property. Moreover, early DNAzymes were essentially 10-23 DNAzymes. Different DNAzymes have different catalytic activities in the presence of different metals, whose ions can produce different biological functions in the body. Therefore, multiple DNAzymes have been widely used in treatment studies. Additionally, DNAzymes should be combined with other treatments to achieve efficient and synergistic treatment. These early problems have been gradually emphasized in recent studies and will be discussed further in subsequent chapters.

## 2.2. Problems Identified during Development

### 2.2.1. Shortcomings of Random-Based Structural Optimization Processes

Antisense therapy usually requires therapeutic agents to be chemically stabilized to resist enzymatic degradation.

DNAzymes also need to be tailored as special chemical structures to play a stable and powerful role in cells, as well as in vivo. However, the development of nucleic acid chemistry has not revealed which stable modification method is the most feasible, or which combination adjustment method is the most effective for DNAzymes (Table 2). Throughout the clinical application or experimental operation, we found that 3'-inversed dT is the most widely used modification method, which has been applied in more than 90% of DNAzyme formulations in clinical studies to date. This is not only because it can resist enzymatic degradation primarily by 3'-exonucleases, but also because it is safe and stable.<sup>[71]</sup> It is more cost-effective than other stable means [phosphorothiate, 2'-O-methylation, and locked nucleic acids (LNAs)] for clinical translation. However, there are many conflicting conclusions regarding the 3'-inversed dT stabilization method, concerning catalytic rate enhancement and antinuclease activity.

Specifically, 3'-inversed dT could increase the catalytic activity of DNAzymes in human serum or plasma from 70 min to 22 h, and oligonucleotides with catalytic activity could be extracted 24 h after cell transfection.<sup>[72]</sup> In addition, Santiago et al. compared the molecular structural integrity of DNAzymes with right or reversed 3'-thymidine orientation after incubation in the serum. DNAzymes of the correct orientation group were almost completely degraded at 24 h, while the inverted orientation was almost unaffected even after 48 h.<sup>[10]</sup> Furthermore, Sun et al. compared the effect of the 3'-modification on the catalytic activity of DNAzymes under different substrate-binding arm lengths.<sup>[73]</sup> They found that reverse thymidine modification decreased catalytic efficiency for the short recognition arm DNAzymes (7 + 7 bp). When the substrate recognition arm was extended to 9 + 9 bp, the relative catalytic efficiency was improved tenfold through reverse base modification. A DNAzyme with an intermediate length (8 + 8 bp) binding arm showed a twofold increase in catalytic efficiency. Thus, 3'-inversed dT base improvement varied depending on the length of the substrate-binding arm. A length of 8–9 bp was found to be optimal in this study. Moreover, the inverted dT at the 3'-ends of DNAzymes have recently been modified to improve resistance to 3' → 5' exonuclease.<sup>[74]</sup> Surprisingly, Kurreck and co-workers showed the opposite conclusion. They found that 3'-inverted-dT modification did not show any potential to improve the catalytic activity of DNAzymes.<sup>[15a]</sup>

This opposite verdict questioned the ability of 3'-end capping inverted dT to increase the catalytic activity of DNAzymes.

Additionally, many other molecular chemotypes, including phosphorothioate linkages,<sup>[75]</sup> 2'-O-methylribonucleotides,<sup>[76]</sup> LNAs,<sup>[77]</sup> and hairpin structure capping,<sup>[78]</sup> have also been investigated for substrate-binding arm<sup>[79]</sup> modification. Notwithstanding, there are many contradictions concerning these modifications.<sup>[14]</sup> For example, phosphorothioates have been used to protect oligonucleotide sequences from degradation. However, after replacing all phosphorothioate binding arms, DNAzymes were completely inactivated in the substrate excess experiment.<sup>[15a]</sup> Moreover, phosphorothioate renders antisense oligodeoxynucleotides toxic<sup>[80]</sup> and immunoreactive<sup>[81]</sup> and increases the affinity for cellular proteins.<sup>[82]</sup>

In addition, Maiti and co-workers<sup>[83]</sup> modified two and four LNA monomers on both ends of the DNAzyme recognition arm and conducted a comparative study on miRNA silencing. The silencing effect of DNAzymes modified with four LNAs was enhanced, compared with that of DNAzymes modified with only two LNAs, which was better than that of unmodified DNAzymes. Subsequently, the group used LNA-modified DNAzymes to inhibit mature and precursor miRNAs,<sup>[84]</sup> and the results confirmed the LNA function. However, Kurreck and co-workers<sup>[15a]</sup> challenged this phenomenon. When four or more LNA nucleotides were introduced at both ends of the substrate-binding arm, the reaction speed of DNAzymes was greatly reduced, probably because of the slow release of the cleaved substrate.

In an example of 2'-O-methyl modification, Kurreck and co-workers<sup>[15a]</sup> found that modifying four or five 2'-O-methyl monomers at the 5'- and 3'-ends of the substrate recognition arm achieved higher activity than that of the LNA-modified DNAzyme. By contrast, Chaput and co-workers<sup>[85]</sup> found that the catalytic activity of DNAzymes modified with 2'-O-methyl monomers was slower than that of LNA replacement, regardless of single turnover or excess substrate conditions.

The reasons for the abovementioned conflicting conclusions, which exist even in current studies,<sup>[85,86]</sup> are that detecting catalytic rates is not comparable owing to the different conditions and priorities adopted by each laboratory. The specific reasons for this may be as follows. 1) Each research group adopted different substrate sequences, and the long sequence formed different secondary structures that hindered the accessibility of DNAzymes. 2) Each group was treated with a different catalytic medium. Thus, researchers are expected to standardize the catalytic media to avoid conflicting results. Moreover, since some studies may involve miRNAs or long-chain mRNAs, the catalytic substrates cannot be the same; thus, the catalytic rates of different substrate sequences should be compared separately.

Modifications to the substrate recognition arm vary, and no study to date has confirmed the most appropriate modification method. By reviewing the development process, it can be determined that 3'-inverted-dT modification inhibits DNAzyme degradation by exonucleases, allowing DNAzymes to function more efficiently in vivo. Nevertheless, more evidence suggests that in addition to exonucleases, endonucleases also play an important role in DNAzyme metabolism,<sup>[87]</sup> while 3'-3'-terminal-linked dT cannot resist endonucleases, such as Exo I or Exo III. Therefore, modifying all DNAzyme nucleotides with different chemotypes aims to solve the enzymatic degradation problem. However, this

idea is difficult to implement. First, even slight modifications of the catalytic DNAzyme core involve a conformational change in the DNAzyme/RNA complex, which often reduces catalytic activity.<sup>[88]</sup> Second, modifications to the substrate-binding arm have several limitations. Overly modified nucleic acids lead to excessive affinity between DNAzymes and substrates, resulting in substrates not being separated from DNAzymes after cleavage, thereby inhibiting catalysis via multiple turnover rates. Moreover, excessive substrate modification causes nucleotides close to the catalytic core to interfere with its conformation.<sup>[15a]</sup> To address these issues, new molecular mechanisms and conformational explorations have been initiated over the past five years, which will be discussed in the next chapters.

### 2.2.2. Lack of Metal Cofactors and Off-Target Concerns

The lack of metal cofactors at the disease site is detrimental to the in vivo activity of DNAzymes. Experiments have clearly indicated that the lack of metal ions is the root cause of low or no DNAzyme activity in vivo.<sup>[89]</sup> At present, the clinical applications of DNAzymes are all based on direct delivery without cofactor supplementation, which may be the main reason for unsatisfactory clinical results.

In addition, DNAzymes can achieve specific substrate gene regulation using accurately designed substrate recognition sequences with accuracy up to a single nucleotide.<sup>[90]</sup> Experiments have also shown that DNAzymes have higher targeting properties than antisense oligonucleotides (ASOs).<sup>[91]</sup> However, some oncogenic or drug-resistant genes not only exist at the disease site, but also play an important role in healthy cells. Indiscriminate regulation in all tissues can lead to significant adverse effects. For example, heat shock protein (HSP) 70 is a cell-stress protein inducing cells to resist high temperatures and damage.<sup>[92]</sup> Its high expression in healthy cells can reduce damage caused by hyperthermia.<sup>[93]</sup> However, high HSP70 expression in cancer cells can lead to tumor resistance to photothermal therapy. Therefore, when DNAzymes are used to regulate HSP70 mRNA expression, healthy and paracancerous tissues should be distinguished.

### 2.3. Solution Exploration

In recent years, scientists have gradually become aware of the abovementioned challenges, including 1) standardization of chaotic catalytic rate research, 2) simultaneous augmentation of catalytic activity and enzyme resistance capacity, 3) supplementation of metal cofactors in target tissues, and 4) improvement of DNAzyme tissue recognizability.

Therefore, the structural stability improvement of DNAzymes has gradually evolved from random modification to mechanistic studies based on theoretical guidance. Moreover, a series of standardized schemes has been proposed to study DNAzyme catalytic rates. Advanced nanocarrier design also realized sufficient metal cofactor supply in diseased tissues and improved the tumor recognition and synergistic therapeutic effect of DNAzymes. Therefore, DNAzyme research has become a completely new field. In the following chapters, we will gradually analyze the



research frontiers of DNAzymes, from their structural design, delivery systems, and stabilization strategies, to their combinations and advanced therapeutic applications.

### 3. Recent Structure Designs for DNAzyme Catalytic Efficiency Augmentation

In the last five years, owing to great progress in the field of nucleic acid chemistry, the number of articles on DNAzyme therapy has increased exponentially. Clinical setbacks have prompted researchers to re-examine the molecular mechanisms and functions of each nucleic acid inside DNAzymes. In addition, the associated company “Sterna Biologicals GmbH & Co. KG” also conducted a second round of financing for DNAzyme therapy, used for novel DNAzyme-based therapy.

Current structural mechanistic studies on DNAzymes can be summarized in two aspects: 1) structural analysis of the substrate-binding arms and 2) functional studies of the nucleotides in the catalytic core.

#### 3.1. Substrate-Binding Domain

Clear concepts have been gradually developed for substrate recognition sequence modifications.<sup>[94]</sup> First, the affinity between the DNAzyme and its target sequence should increase, but DNAzyme separation from the cleaved substrate should not be affected. Second, the ability of biological enzymes to degrade DNAzymes must be reduced.

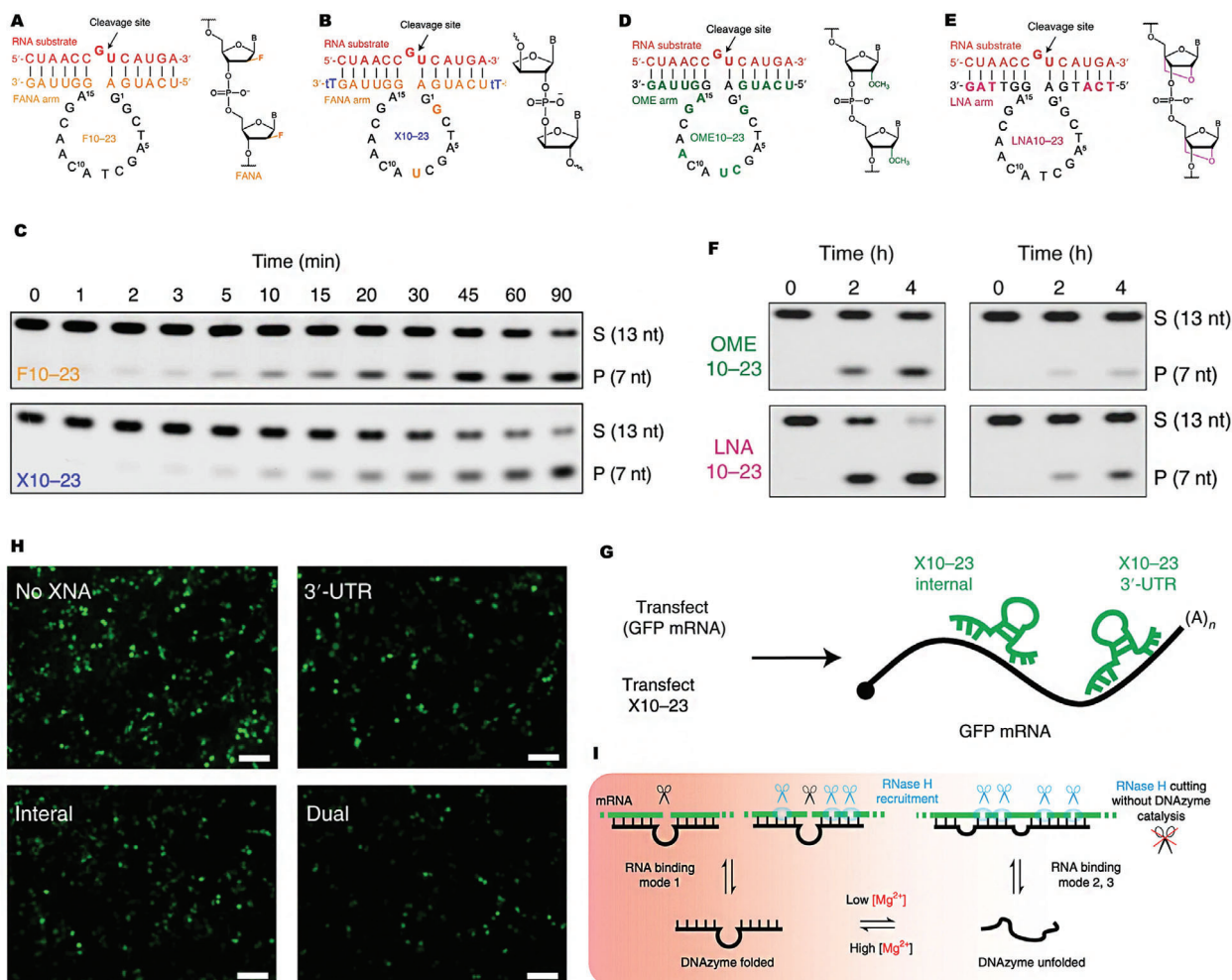
In 2021, 10-23 DNAzyme was systematically modified by Chaput and co-workers to balance binding kinetics with multiple turnover catalytic effects.<sup>[85]</sup> In this study, 2'-fluoroarabino nucleic acid (FANA), a xeno-nucleic acid (XNA)<sup>[95]</sup> with completely different physical and chemical properties from those of natural DNA and RNA, were utilized to replace all nucleotides in the substrate chain, abbreviated as F10-23 (Figure 2A). FANA represents the substitution of the 2'-hydroxy group of 2'-deoxyarabino with a fluorine atom,<sup>[96]</sup> which can increase the affinity between DNAzyme and target RNA. Furthermore,  $\alpha$ -L-threofuranosyl nucleic acid (TNA) was added to the 5'- and 3'-terminal positions to protect the DNAzyme from nuclease digestion, abbreviated as X10-23 (Figure 2B). For TNA, the natural five-carbon ribose in RNA was replaced with a four-carbon threose that was completely resistant to DNA- and RNA-degrading enzymes.<sup>[97]</sup> The activities of F10-23 and X10-23 were 50 times higher than that of the parent DNAzyme, when the substrate RNA concentration was tenfold higher than that of the DNAzyme. This indicated that F10-23 and X10-23 had important structural differences between their precatalytic and postcatalytic states compared to the parent 10-23 DNAzyme.

Due to the TNA modification, X10-23 could resist high levels of DNA nuclear enzymes, compared with F10-23, which clearly suggested that X10-23 possessed markedly enhanced biostability (Figure 2C). Among other nucleic acid modifications, 2'-O-methyl ribonucleotides (OME) and LNA-modified nucleic acids have also received significant attention owing to their enhanced activity and biological stability. Therefore, OME10-23 (Figure 2D) and LNA10-23 (Figure 2E) were also prepared, and their catalytic

efficiencies were compared. Under all test conditions, LNA10-23 was significantly faster than OME10-23, but it was slower than the abovementioned X10-23 under multiturnover conditions (Figure 2F). Finally, XNAzyme was selected to verify gene silencing in the green fluorescent protein (GFP)-transfected human embryonic kidney 293 (HEK293) cells (Figure 2G). The gene silencing efficiency was the highest when multiple sites of GFP mRNA were targeted (Figure 2H). In summary, this achievement represents an important advance in the design of therapeutic DNAzymes in clinical research.<sup>[98]</sup> The target gene shearing efficiency and degradation resistance were simultaneously improved by chemical modification of the DNAzymes.

Interestingly, a scientific debate published in *Nature Chemistry* in 2022<sup>[86a,c]</sup> between Spitale and Chaput from the University of California and Taylor and Holliger from the University of Cambridge based on the abovementioned work is expected to exert a standardized and clear driving force for overall therapeutic DNAzyme-based catalytic study. Taylor and Holliger questioned the core argument of this research from three perspectives. First, RNase H may be the primary cause of targeted mRNA degradation after full replacement of the substrate recognition arm with LNA, as reports show that up to five complex chains of FANA and DNA can enhance target RNA degradation through RNase H.<sup>[99]</sup> In addition, DNAzymes are mainly found in the unfolded state due to the lack of metal coenzyme factors in the in vivo physiological environment;<sup>[100]</sup> therefore, FANA-modified DNAzymes may very likely perform imperfect pairing with the target RNA, leading to RNase H recruitment (Figure 2I). Second, Taylor and Holliger repeated the catalytic effect of X10-23 DNAzymes and found that their catalytic rate was not as high as that mentioned in ref. [85]. They suspected that the authors used unrealistic short-chain RNA as the substrate for the experiment. In current research on therapeutic DNAzymes, researchers conveniently use short-chain RNA sequences as substrates, which do not have secondary structures.<sup>[101]</sup> This method can determine the success of the modification by comparing the catalytic rates of modified and unmodified DNAzymes. However, DNAzymes encounter RNA sequences that have stable secondary structures in vivo. The complex secondary structure hinders DNAzymes from accessing RNA, thereby reducing their catalytic rate. Because the targets studied in each study were different, the RNA sequences and secondary structures were incompatible, which explains why the catalytic rates between different groups were difficult to compare, even with the same DNAzyme. Third, Taylor and Holliger proposed that LNA-modified DNAzymes are difficult to degrade using standard DNase treatment steps and will continue to cleave the substrate sequence during quantitative polymerase chain reaction (qPCR). Treating qPCR as a single reading would therefore result in a false positive.<sup>[102]</sup>

Chaput and co-workers immediately replied to all arguments. The authors pointed out that X10-23 only achieved gene silencing against wild-type and G12V mutant Kirsten rat sarcoma virus (KRAS) mRNA in a previously verified allele-specific gene silencing experiment, and there was no off-target effect.<sup>[86b]</sup> If RNase H mediates mRNA silencing, similar genes should also be silenced in large quantities. In addition, the authors replied that the FANA oligonucleotide could increase the potency of RNase H only in the specific case of ASO construction using the gapmer method.<sup>[99]</sup> Thus, the central DNA region should act as the binding site for



**Figure 2.** Comparison of different modification methods of DNAzymes and catalytic effects. A,B) Structure of F10-23 and X10-23 DNAzymes. C) Comparison of catalytic rates of F10-23 and X10-23 DNAzymes. D,E) structure of OME10-23 and LNA10-23 DNAzymes. F) Representative polyacrylamide gel electrophoresis (PAGE) gels showing RNA cleavage activity of OME10-23 and LNA10-23 DNAzymes under steady-state (left) and multiple turnover conditions (multiple-turnover). G) Schematic representation of cells transfected with GFP mRNA and DNAzymes simultaneously. H) Gene silencing results in different splicing situations. A–H) Adapted with permission.<sup>[85]</sup> Copyright 2021, Springer Nature. I) Schematic diagram, proposing that at low physiological concentrations of  $Mg^{2+}$ , and when the target RNA is longer and more structured, DNAzymes achieve RNase-H-mediated RNA cleavage through RNA interactions with residues sequestered in their catalytic core, in contrast to DNAzyme's catalytic activity is irrelevant. Adapted with permission.<sup>[86c]</sup> Copyright 2022, Springer Nature.

RNase H, whereas the flanking FANA fragment should serve to increase the affinity of ASO for mRNA, rather than FANA acting as the binding site for RNase H.

Furthermore, the cleavage sites may be different even for the same substrate strand. Hence, different DNAzyme accessibility to the substrate strand will influence catalytic efficiency. Chaput and co-workers claimed that Taylor and Holliger did not provide sufficient operational data, which made their comparison inappropriate. However, the authors acknowledged and agreed with the views of Taylor and Holliger regarding the selection of the negative blank control. In detail, it would be better to change a single base in the catalytic ring instead of using core inverted X10-23i as the control group. Chaput and co-workers further agreed that different cells express different levels of RNase H. Therefore, cell-free experiments are needed to standardize and simulate intracellular environments.

Overall, based on the debate between Chaput and co-workers and Taylor and Holliger, to eliminate conflicting conclusions, researchers are expected to use long-chain RNA instead of short-chain RNA fragments to simulate real environments in future experiments. In addition, the catalytic medium should be unified, including the metal content and nuclease addition. The RNase-H-mediated antisense effect should be fully considered, and the silencing efficiency of the target RNA should be verified using various means.

### 3.2. Catalytic Core Modifications with Theoretical Guidance

There are numerous optimization strategies for the catalytic loop of DNAzymes.<sup>[103]</sup> Conventional practices focus on improving DNAzyme resistance against nucleases, thereby improving

bioavailability,<sup>[104]</sup> or replacing the functional groups on multiple nucleic acids to improve catalytic activity.<sup>[15a,105]</sup> For example, the 6-amino group in adenine can be replaced or stabilized as a general acid or base to participate in catalytic reactions. However, functional modification of DNA residues in the catalytic loop of DNazymes may lead to self-defeating effects. Substitution at the C10, G14, and A15 positions led to low DNzyme activity (20–30%).<sup>[85]</sup> Because optimization of the DNzyme catalytic core is different from its substrate recognition arms, enhanced DNzyme stability should be considered, as well as the systematic study of the catalytic ring conformation and its interaction with cofactor metal ions.

The development of DNzymes based on the abovementioned random optimization<sup>[106]</sup> or systematic screening<sup>[107]</sup> has led to progressive breakthroughs. However, despite the satisfactory catalytic efficiency of DNzymes *in vitro*, clinical cases of successful treatment are still rare to date,<sup>[13b]</sup> which is related to the decreased catalytic activity in the cellular environment and deficient knowledge of the underlying mechanism. Rational sequence design and structural modification strategies based on scientific theoretical guidance can expedite the development of DNzyme technology.<sup>[108]</sup> Therefore, it is important to fully understand the basis of DNzyme-mediated catalytic processes, including their potential molecular structures, dynamic processes, and metal cofactor interactions.

At present, the crystal structures of various RNA-cleaving DNzymes have been resolved.<sup>[109]</sup> These structural studies are important, because they reveal a reasonable catalytic mechanism for DNzymes, the role of metal cofactors, and the necessary nucleotides in the DNzyme sequence.<sup>[9a]</sup> Among them, the function of each nucleotide at the catalytic ring site of the two best-known RNA-cleaving DNzymes (10-23 and 8-17) has been studied deeply and systematically, which can improve the rational design of DNzymes to ensure high catalytic efficiency *in vivo*. Here, the numerical designation 10-23 indicates the 23rd clone in the 10th round of DNase selection experiments, and 8-17 denotes the 17th clone in the 8th round.<sup>[110]</sup>

Specifically, cationic cofactor binding to the active sites of 8-17 DNzyme (**Figure 3A**) and substrate cleavage catalysis were studied in 2017.<sup>[109b]</sup> By observing the crystal structure of the 8-17 DNzyme Dz36/substrate complex, Gan and co-workers found that G+1 and G–1 were kinked at the substrate cleavage site and paired with T1 and A14 on the catalytic ring, respectively (**Figure 3B**). This structure could fix the orientations of G+1 and G–1 and promote RNA cleavage. When metal cofactors participated in the catalytic reaction, the Pb<sup>2+</sup> cofactor could not induce additional DNzyme folding, whereas Mg<sup>2+</sup> and Zn<sup>2+</sup> could induce additional folding, resulting in lower catalytic rates.<sup>[111]</sup> Furthermore, the general acid–base catalytic mechanism of 8-17 DNzymes was elucidated. The nucleobase of the G13 residue may act as a general base to deprotonate the 2'-OH of G–1 to attack the 3'-phosphate of G–1 (**Figure 3C**). The authors showed that the N1 atom of G13 facilitates DNase catalysis after observing a significant decrease in DNzyme activity through methylation at the N1 position. At the same time, the pK<sub>a</sub> of the water molecule decreased after it coordinated with the Pb<sup>2+</sup> cofactor,<sup>[112]</sup> and it could be used as a general acid to provide a proton for the O5' of G+1 to leave, thus promoting the cleavage of the RNA target (**Figure 3D**). Overall,

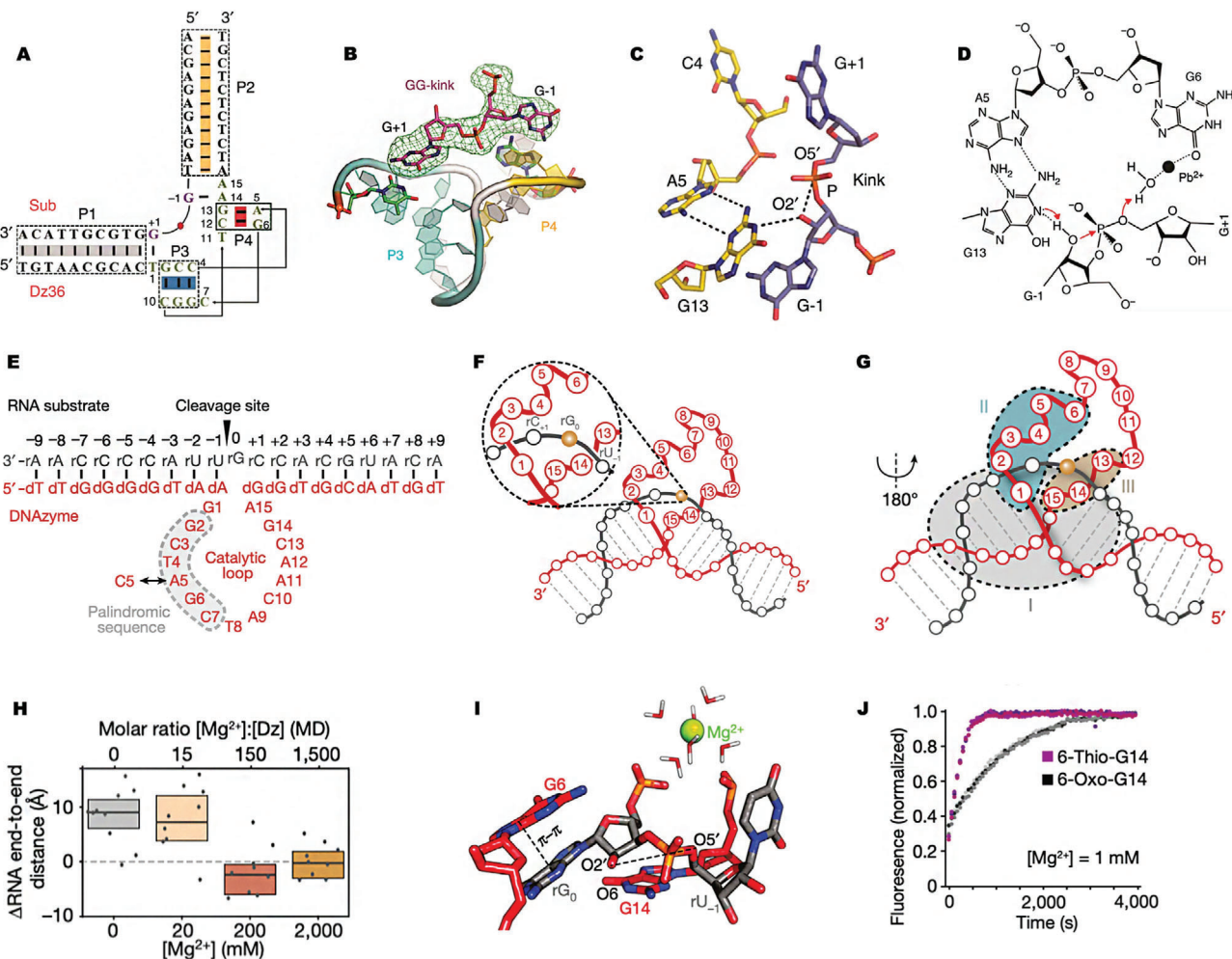
a clear understanding of each important nucleotide function within the catalytic ring will facilitate the design of biopolymers, providing new solutions to enhance DNzyme catalytic activity.

Fast forward to 2022, Etkorn and co-workers first performed NMR-based time-resolved structural analysis of 10-23 DNzyme.<sup>[109a]</sup> In this study, the molecular structure, conformational transformation, and coordinate region of metal ions in the precatalytic DNzyme–RNA complex were studied, revealing the factors affecting DNzyme-mediated catalysis. First, as depicted in **Figure 3E**, the 10-23 DNzyme contained 15 nucleotide catalytic loops. The 5'-adenine of the catalytic ring (A5) was replaced with cytosine to avoid the risk of catalytic loop dimerization caused by the palindromic sequence<sup>[109d]</sup> and to enhance NMR accessibility. Temperature-dependent NMR experiments revealed that the catalytic DNzyme ring was partially fixed. The 5'-end (C3–C7) was fixed to facilitate catalysis, whereas the 3'-end (T8–C13) possessed plasticity to facilitate conformational transformation. This unexpected molecular structure allowed the complete exposure of the substrate cleavage site to the catalytic ring region (**Figure 3F**). Moreover, three metal-binding sites were identified in the catalytic loop. Binding site I can promote cleavage-site alignment, site II responds to catalytic activation, and site III is responsible for C13 and G14 activation to improve 10-23 DNzyme catalytic activity (**Figure 3G**). According to the Brønsted–Lowry acid–base theory and microsecond-long all-atom molecular dynamics simulations, the authors found that Mg<sup>2+</sup> reduced the basicity of G14 by reducing the electron density on the purine ring, thereby blocking the interaction between G14 and the cleavage site (**Figure 3H,I**). The 6-thiol modification of G14 resulted in a sixfold increase in cleavage rate (**Figure 3J**). In conclusion, DNzyme optimization requires special modification of the three metal-binding sites.

Different DNzymes have different catalytic ring structures and coordination states of metal cofactors. For example, compared with another DNzyme (9DB1), studied by Orozco and co-workers in 2019,<sup>[113]</sup> only two Mg<sup>2+</sup> binding sites were found, while the abovementioned 10-23 DNzyme had three binding sites. The two DNzymes exhibited catalytic rings with completely different spatial structures. Therefore, modification of the catalytic ring should depend on our understanding of the functionality of each nucleotide in the catalytic loop of each DNzyme. This design optimization pattern based on nucleic acid function is superior to traditional random optimization of DNzymes.

#### 4. Recent Delivery Strategies for DNzyme Catalytic Improvement

In addition to chemical modifications, nanomaterial-based strategies are another solution to overcome the shortcomings of DNzymes. Currently, DNzymes are synergized or protected considerably by other materials, including organic or inorganic constituents. These carrier construction approaches aim to improve DNzyme stability and loading degree and realize synergistic therapeutic strategies. Hence, in this chapter, combinations of various materials with DNzymes are discussed from the perspective of drug delivery system construction.



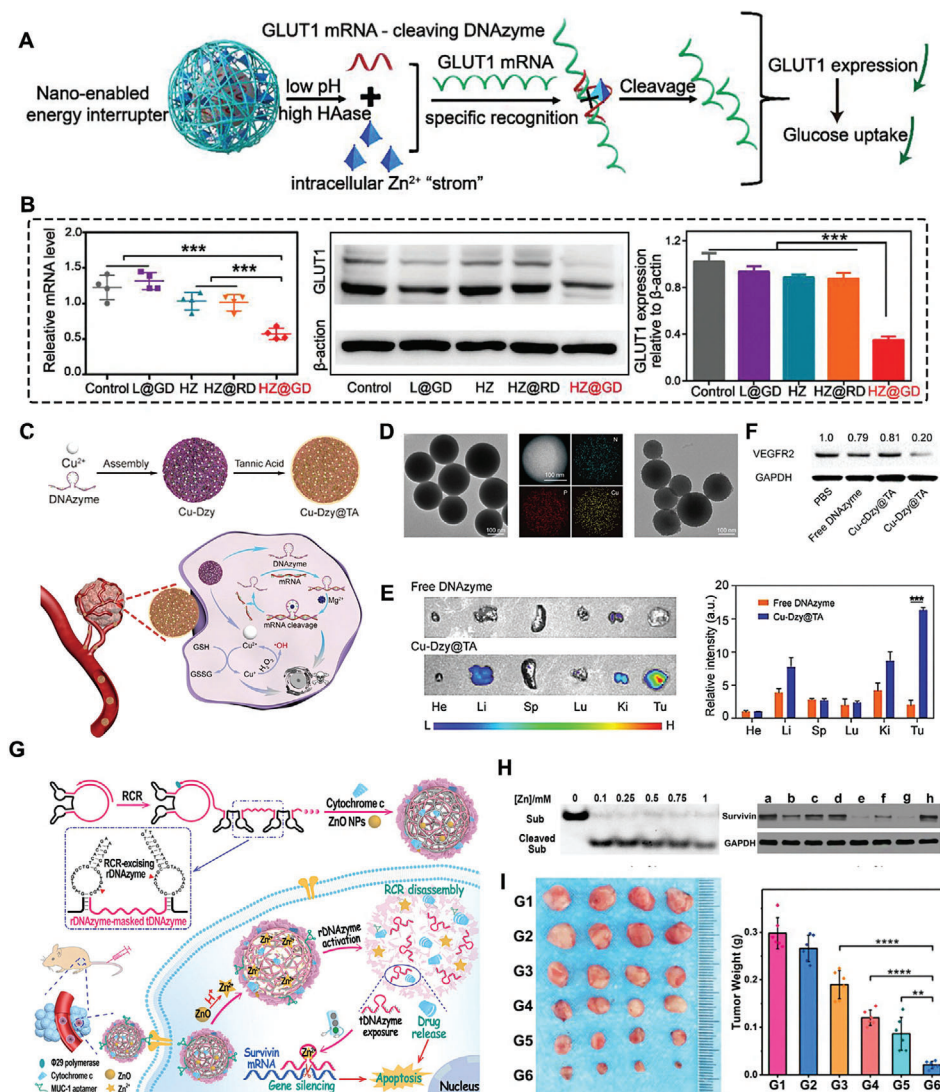
**Figure 3.** Mechanistic studies on the catalytic core conformation of DNAzymes. A) Sequences of DNAzymes and substrates and observed secondary structures. B) Mimics of the catalytic core and GG kink based on the DNAzyme–Pb<sup>2+</sup> complex structure. C) Coordination simulation of Pb<sup>2+</sup> with catalytic water in the DNAzyme/Pb<sup>2+</sup> structure. D) Mechanistic predictions regarding the cleavage of RNA substrates by 8–17 DNAzymes. A–D) Adapted with permission.<sup>[109b]</sup> Copyright 2017, Springer Nature. E) Schematic diagram of 10–23 DNAzymes for targeting mRNA. F) Simplified representation of the precatalytic complex, highlighting the association of the catalytic core with the catalytic site. G) Simplified view of three metal-ion binding regions. H) Metal ions activate DNAzymes by condensing the DNAzyme structure and bringing the catalytic loop into an active conformation. I) Snapshot of molecular dynamics (MD) simulation showing a plausible cleavage mechanism involving the interaction of G 14 with the O2' atom of rG<sub>0</sub>. J) Modification of 6-thio-G 14 significantly increases the activity in the presence of Mg<sup>2+</sup>. E–J) Adapted with permission.<sup>[109a]</sup> Copyright 2021, Springer Nature.

#### 4.1. Cofactor-Incorporated Delivery Strategies

Owing to the special metal-ion activation characteristics of DNAzymes, an increasing number of studies have focused on the smart utilization of the corresponding metal cofactors. These metal cofactors can be fabricated as the material components of the nanocarrier and realize the corresponding ion-based therapy.

Zinc ions are effective in inhibiting glycolysis and achieving hunger treatment in tumors.<sup>[114]</sup> In addition, DNAzymes using zinc ions as an activation cofactor have also been widely used.<sup>[115]</sup> Therefore, silencing glucose-supplied genes with DNAzymes and using zinc as the metal cofactor and nanocarrier material is an effective synergistic therapeutic strategy. Shi and co-workers used hyaluronic-acid (HA)-encapsulated ZIF-8 (ZIF = zeolitic imidazolate framework) as a drug delivery vehicle and encapsulated DNAzymes that could cleave glucose transporter 1 (GLUT1)

mRNA<sup>[116]</sup> (Figure 4A). This system could actively target tumor cells through CD44-mediated enhanced uptake. After endocytosis, the released Zn<sup>2+</sup> could mediate tumor-specific “Zn<sup>2+</sup> interference,” with a strong inhibition of glycolysis through nicotinamide adenine dinucleotide (NAD<sup>+</sup>) downregulation and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) inactivation. Moreover, Zn<sup>2+</sup> interference could reduce GLUT1 expression by specifically activating DNAzyme catalytic cleavage (Figure 4A). Experimental results showed that the final composite, HA-modified ZIF-8 nanosystem loaded with DNAzyme (HZ@GD), can cut off the supply of glucose and further achieve energy depletion in the tumor system (Figure 4B). The nanosystem could achieve 80.8% tumor growth inhibition *in vivo* without systemic toxicity. This study validated an intriguing therapeutic platform that enables an ion-interference-induced starvation strategy to effectively treat tumors. On the other hand, Zn<sup>2+</sup> has also been



**Figure 4.** Divalent metal cofactors for functional carrier construction and synergistic therapy. A) GLUT1 mRNA cleaving DNAzymes combined with  $Zn^{2+}$  nanocarrier. B) The silencing effect of GLUT1 mRNA level for the nanoenabled DNAzymes. A,B) Adapted with permission.<sup>[116]</sup> Copyright 2022, Wiley-VCH. C) DNAzymes synergized with  $Cu^{+}$ . D) TEM picture of DNAzyme/ $Cu^{+}$  composite. E) The biodistribution of the nanosystems. F) VEGF-R2 inhibition effect for different treatment groups. C–F) Adapted with permission.<sup>[126]</sup> Copyright 2021, Wiley-VCH. G) Functional DNAzymes protected by secondary DNAzymes for stimulated carrier building and synergistic therapy. H) substrate strand and target survivin gene depletion effect. I) Tumor ex vivo photos and tumor mass. G–I) Adapted with permission.<sup>[131]</sup> Copyright 2021, Wiley-VCH.

reported as an intracellular ATP scavenger to reduce the expression of HSPs<sup>[117]</sup> or the tumor drug resistance gene P-glycoprotein (P-gp).<sup>[118]</sup> In addition, the acidic/hypoxic response properties of  $Zn^{2+}$ -based ZIFs have been widely investigated.<sup>[119]</sup> Therefore, we believe that multistage gene/ion synergistic therapy realized by combining DNAzymes with other  $Zn^{2+}$  functions will expand the construction of therapeutic systems.

$Mn^{2+}$  has the advantage of an excellent chemodynamical effect for converting endogenous  $H_2O_2$  into toxic  $\bullet OH$ . Moreover,  $MnO_2$ -based nanomaterials have been widely prepared to obtain glutathione (GSH)-responsive nanocarriers.<sup>[120]</sup> Therefore, DNAzymes can be integrated with  $MnO_2$  nanomaterials for  $Mn^{2+}$ -activated responsive drug delivery and synergistic therapy.<sup>[121]</sup> Peng et al. incorporated two  $Mn^{2+}$ -triggered

DNAzymes within  $MnO_2$ -nanosheet-based systems specifically activated by intracellular miRNA-155 and telomerase biomarkers for different therapeutic functions, including antitumor angiogenesis and proapoptosis mRNA gene regulation.<sup>[122]</sup> The DNAzymes adsorbed on the complex were released specifically in a tumor environment with sufficient metal cofactor  $Mn^{2+}$ . Meanwhile, EGR-1 and survivin mRNA were knocked out after DNAzymes were unlocked, and a double-gene synergistic therapy was realized. Simultaneous fluorescence imaging was achieved by designing DNAzymes with locker structures.

Tumor-specific release of  $Cu^{+}$  ions can efficiently catalyze  $H_2O_2$  to produce  $\bullet OH$  through a Fenton-like reaction.<sup>[123]</sup> In addition, the released  $Cu^{+}$  ions deplete GSH within cells, thus protecting  $\bullet OH$  from being removed, which greatly improves

chemokinetic therapy efficiency.<sup>[124]</sup> Therefore, Cu<sup>+</sup> ions have also been widely combined with DNAzyme-mediated gene therapy.<sup>[125]</sup> Zhao and co-workers constructed a hybrid nanostructure with an ultrahigh loading capacity, which consisted only of DNAzyme and Cu<sup>2+</sup><sup>[126]</sup> (Figure 4C). Transmission electron microscopy (TEM) characterization revealed that the nanoparticles had a smooth, spherical shape (Figure 4D). After codelivery of DNAzyme and Cu<sup>2+</sup> to cancer cells, GSH reduced the released Cu<sup>2+</sup> to Cu<sup>+</sup>. A large amount of Cu<sup>+</sup> can catalyze endogenous H<sub>2</sub>O<sub>2</sub> to form cytotoxic •OH radicals for chemodynamic therapy. The experimental results showed that the system effectively accumulated in tumors and showed an amplified cascade antitumor effect (Figure 4E). Later, DNAzymes catalyzed the cleavage of VEGF-R2 mRNA to achieve gene therapy (Figure 4F).

Wang and co-workers have also been dedicated to this DNAzyme/ion synergistic strategy in recent years. In addition to the previously mentioned synergistic therapy combining DNAzymes with Mn<sup>2+</sup>,<sup>[127]</sup> systems for other metals have also been extensively constructed. They first utilized ZIF-8 nanoparticles to carry out DNAzyme-based gene therapy with a Zn<sup>2+</sup> cofactor self-sufficient effect.<sup>[128]</sup> Chu and co-workers adopted ZIF-8 nanoparticles as DNAzyme carriers, but they were only used for intracellular miR-21 detection.<sup>[129]</sup> In the same year, a Zn<sup>2+</sup> self-sufficient DNAzyme-driven drug delivery system was constructed.<sup>[130]</sup> This system consisted of a DNAzyme-based DNA scaffold fabricated through rolling circle amplification (RCA) guidance, and pH-responsive ZnO nanoparticles were encapsulated within the scaffold. Furthermore, the aptamer sequence was encoded into the RCA template to promote effective vector delivery to the cancer cells. Under stimulation by the tumor/lysosome acidic microenvironment, ZnO can be dissolved into Zn<sup>2+</sup>, which can be further used as a DNAzyme cofactor. Recently, this group established a compact DNAzyme nanoplatform for multifunctional intelligent gene therapy.<sup>[131]</sup> The delicate DNAzymes were temporarily masked by the other self-activated DNAzymes through RCA, so they could remain inactive in physiological liquid, but active under stimulation. As depicted in Figure 4G, nanocapsules can accumulate specifically in cancer cells through multivalent tumor-anchored aptamer chains, where they are activated sequentially in response to cascade DNAzyme activation via intelligent stimulation for the final DNAzyme-mediated gene silencing. Ultimately, the substrate strand and target survivin gene were efficiently depleted (Figure 4H). After *in vivo* treatment, the tumor was significantly suppressed (Figure 4I).

The combined application of metal ions has become a popular research topic.<sup>[123]</sup> Different metal ions can accelerate the catalytic rate of DNAzymes and realize multi-ion-based combination therapy. Zhao et al. developed a synergistic preparation by combining DNAzyme with a Fe/Mn-multimetal (FMM)-based metal-organic framework (MOF)<sup>[132]</sup> (Figure 5A). The FMM MOF enabled efficient delivery of DNAzymes against biodegradation in systemic circulation. Interestingly, once ingested by cancer cells, the multifunctional FMM nanoplates released Fe<sup>2+</sup> under H<sup>+</sup>/GSH stimulation and achieve chemokinetic therapy via the Fenton reaction. At the same time, FMM nanoplates also exhibited photothermal conversion ability, which can improve the overall treatment through the photothermal effect. Subsequently, DNAzymes can target the Twist gene to achieve en-

hanced gene silencing under Mn<sup>2+</sup> catalysis to inhibit tumor growth and metastasis (Figure 5B). Thus, Mn<sup>2+</sup> and Fe<sup>2+</sup> could be significantly released under pH = 5.5 and GSH conditions using atomic absorption spectrometry as a testing method, as shown in Figure 5C. The representative images of the lungs collected from 4T1-tumor-bearing mice show that the final formulation (FMM@Dz) could effectively inhibit cancer cell lung metastasis under laser irradiation. Western blot results also confirmed that the final preparation effectively spliced the Twist gene to achieve tumor proapoptosis (Figure 5D,E).

## 4.2. DNAzyme-Integrated DNA-Functional-Material-Based Delivery Strategies

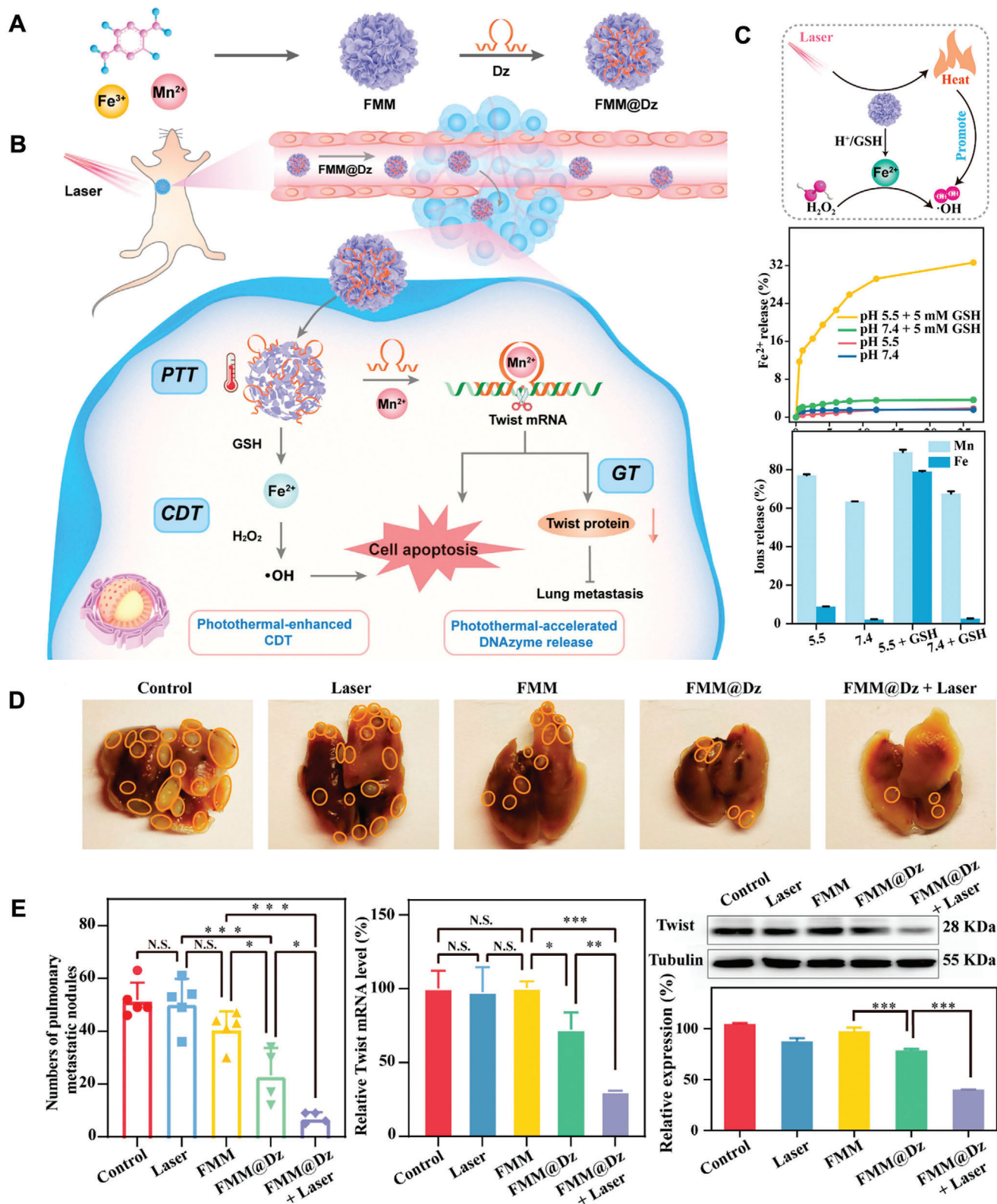
Therapeutic DNAzymes with RNA-depleting effects are versatile building blocks for the construction of sophisticated biological machineries. Combination strategies for DNAzymes with other materials form the basis of synergistic therapies. Among the different materials, the combination of DNAzymes with pure-structural DNA units is simpler and more unique. Therefore, the method of assembling DNAzymes with pure DNA units was first discussed in this section. Functional augmentation strategies for DNAzyme-incorporated DNA functional materials were further illustrated, including RCA, catalytic hairpin assembly (CHA), and toehold-mediated strand displacement.

### 4.2.1. DNAzymes Combined with Pure Structural DNA

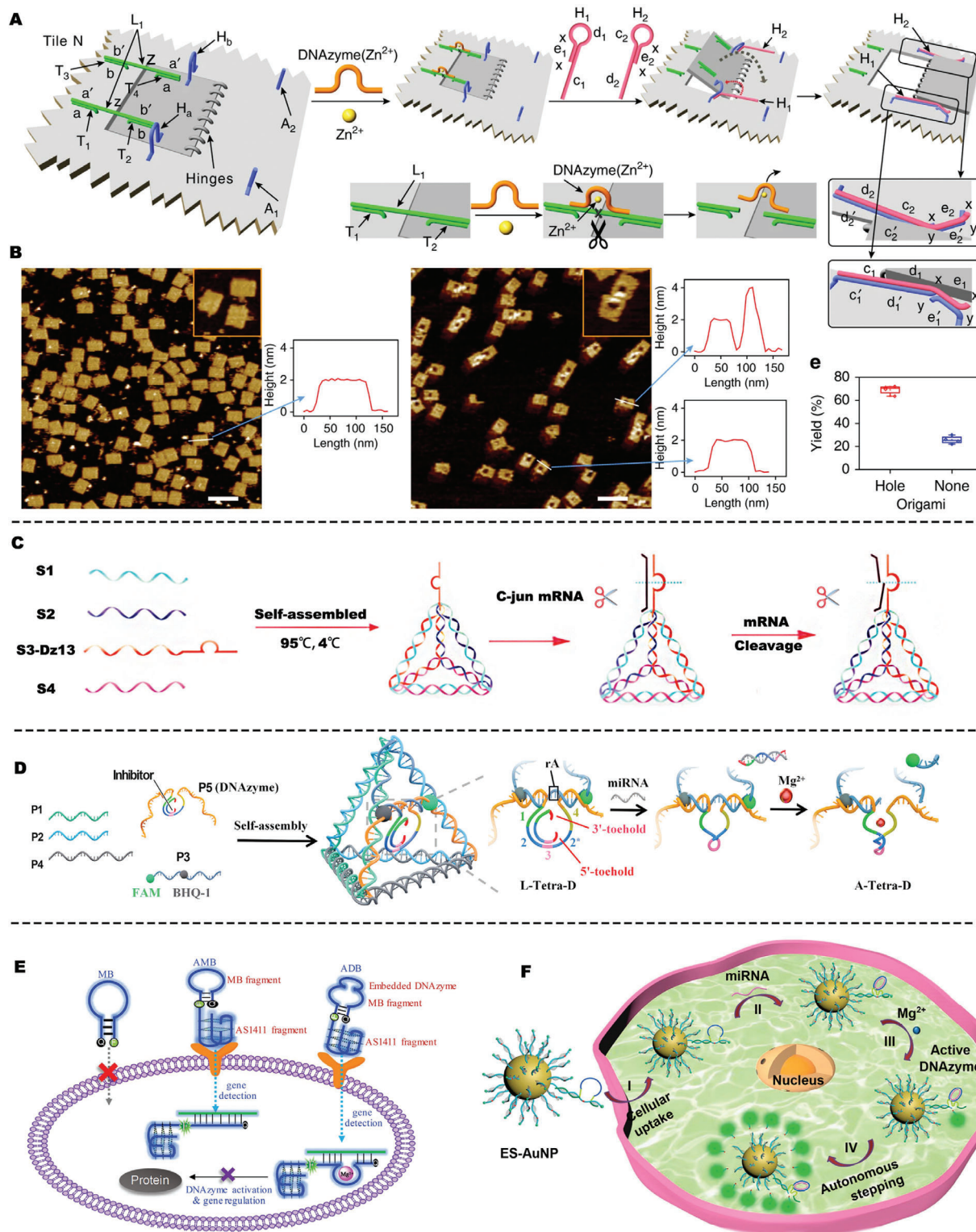
DNAzymes can be combined with structural DNA constituents using the origami technique<sup>[133]</sup> for geometric DNA structures,<sup>[134]</sup> aptamers,<sup>[135]</sup> and other DNA units, such as DNA walker, DNA tweezers.<sup>[136]</sup>

DNA origami technology with accurate spatial addressing and rich site-marking abilities<sup>[137]</sup> has become an ideal platform for the design of custom biological machines.<sup>[138]</sup> Previously, nontherapeutic DNAzymes, such as hemin/G-quadruplex DNAzymes, have been combined with DNA origami technology to construct specific switches.<sup>[139]</sup> Furthermore, Willner and co-workers combined RNA-cleaving DNAzymes as metal-ion-responsive unlocking tools with origami technology to prepare switchable nanocavities,<sup>[140]</sup> as shown in Figure 6A. The lid in the center of the DNA nanosheet (Tile N) was fixed with two L1 strands to form a closed structure. In the presence of Zn<sup>2+</sup>, the system could mediate L1 chain cleavage by DNAzymes to achieve unlocking. Subsequently, the lid can be lifted using helper chains H1 and H2, enabling the formation of voids. Atomic force microscopy (AFM) clearly shows that the lid is pulled open, and the void formation rate is above 60% (Figure 6B). Therefore, combining DNAzymes with DNA-origami-technology-based drug delivery systems<sup>[141]</sup> will enable the construction of nanoplatforms with different gene- or metal-ion-responsive abilities.

Therapeutic DNAzymes have been used to construct tetrahedral DNA nanotherapy systems.<sup>[142]</sup> First, DNAzymes are not efficient in endocytosis, so the membrane permeability of DNA tetrahedrons provides a new strategy for the intracellular transport of DNAzymes.<sup>[143]</sup> Subsequently, a DNAzyme, which cleaves c-Jun mRNA, was added at the 5'-end of the single-stranded DNA tetrahedrons to reduce c-Jun gene expression and inhibit the growth



**Figure 5.** DNAzymes together with dual-metal-based gene/ion therapy. A) DNAzymes combined with two metal-contained MOF systems. B) After endocytosis, the therapeutic mechanism for PTT, PDT, and gene-based triple synergistic therapy. C) The release profile for different metal ions. D) The represented lung pictures of different groups after mice were sacrificed. E) The PCR and Western Blot results for relative twist mRNA level. A–D) Adapted with permission.<sup>[132]</sup> Copyright 2022, Wiley-VCH.



**Figure 6.** DNAzyme-incorporated DNA functional materials. A) Schematic illustration of Zn<sup>2+</sup>-dependent DNAzyme as an unlocking catalyst to form nanopenors in origami. B) AFM images correspond to the locked configuration of the origami tile as well as the unlocked origami tile. A, B) Adapted with permission.<sup>[140]</sup> Copyright 2019, Springer Nature. C, D) Mechanism of action of two DNA tetrahedral nanomachines containing DNAzymes. C) Adapted with permission.<sup>[142]</sup> Copyright 2019, American Chemical Society. D) Adapted with permission.<sup>[144]</sup> Copyright 2022, Elsevier Ltd. E) Schematic representation of aptamer/DNAzyme complexes for cancer-related gene detection and regulation. Adapted with permission.<sup>[145]</sup> Copyright 2019, Royal Society of Chemistry. F) DNAzyme-mediated assembly of DNA walkers and their intracellular manipulation for miRNA imaging. Adapted with permission.<sup>[16]</sup> Copyright 2021, American Chemical Society.



of squamous cell carcinoma (Figure 6C). Therapeutic DNazymes could be efficiently delivered through the tetrahedral DNA structure, and cell proliferation was significantly reduced. In addition to target mRNA silencing, researchers have reported that DNazymes, which bind to DNA tetrahedrons, could simultaneously detect miRNA content and regulate gene expression in cells.<sup>[144]</sup> Specifically, the P1, P2, P4, and P5 chains constitute the basic backbone of the DNA tetrahedron, and P5 contains the DNzyme sequence, which can cut the P3 sequence (Figure 6D). This operation can separate the fluorescein amidites (FAM) fluorophore on the P3 sequence from the Black Hole Quencher-1 dye (BHQ1) quencher to realize fluorescence imaging. An important mechanism of this imaging is that DNazymes contain a miRNA-responsive inhibitor sequence, which triggers fluorescence through miRNA activation and silences miRNA to achieve gene therapy.

DNazymes can also conjugate with aptamers through base pairing,  $T_4$  ligation, or sequence integration to enhance the antinuclease effect and improve the tumor-targeting capacity.<sup>[145]</sup> After reasonably designing the sequence structure and forming the catalytic unit, the DNzyme/aptamer complex can monitor tumor-related genes by generating detectable fluorescence signals and regulating gene expression at the mRNA and protein levels. Zhou and co-workers combined aptamers with DNazymes to achieve receptor-mediated enhanced DNzyme delivery to cells (Figure 6E). Simultaneously, cleavage of the target gene was mediated by  $Mg^{2+}$  ions. Furthermore, DNzyme activation upon binding to substrate target sequences enables fluorescence imaging.<sup>[145]</sup> Shi and co-workers designed a round aptamer (Sgc8)–DNzyme (EtNa) complex to achieve local programmed death-ligand 1 (PD-L1) inhibition. This method could significantly improve DNzyme stability and tumor targeting.<sup>[146]</sup> In contrast to the DNA sequence integration method adopted by Zhou and co-workers, Shi and co-workers utilized complementary base pairing and the  $T_4$  ligation strategy. These methods represent basic design ideas for combining DNazymes with DNA materials.

Many other small molecular machines, such as DNA walkers, tweezers, switches, robots, gears, and motors,<sup>[147]</sup> are available to construct DNA biomaterials. The combination of these small components has powerful biological functions, especially in DNA walkers, whose cascade signal enhancement through autonomous movement is considered a powerful signal amplification strategy. Wu and co-workers used a DNzyme-integrated DNA walker system to detect abnormal miRNA expression,<sup>[16]</sup> as shown in Figure 6F. In this study, DNzyme machines were combined with AuNPs. Notably the serum resistance of the DNzyme walker system increased fivefold, and the catalytic activity increased more than eightfold when a circular convex protection structure was introduced in the middle of the DNzyme catalytic core. This protective structure was designed based on the miRNA-21 recognition system, which facilitated the ability to image miRNAs far beyond commercial transfection reagents and the well-known fluorescence in situ hybridization (FISH) technique. Similarly, the system may also achieve miRNA-21 silencing; however, in this work, the authors did not mention this investigation. Another example of using a 3D DNzyme-based DNA walker nanoprobe structure is to image amyloid  $\beta$  oligomers ( $A\beta$ ) in living cells and in vivo. The presence of  $A\beta$

can trigger the DNzyme to walk through the substrate chains, labeled with a fluorophore (named "TAMRA"). The TAMRA-containing substrate strands were modified on the AuNP surface (quencher); hence, substrate cleavage could recover the fluorescence signal.<sup>[148]</sup>

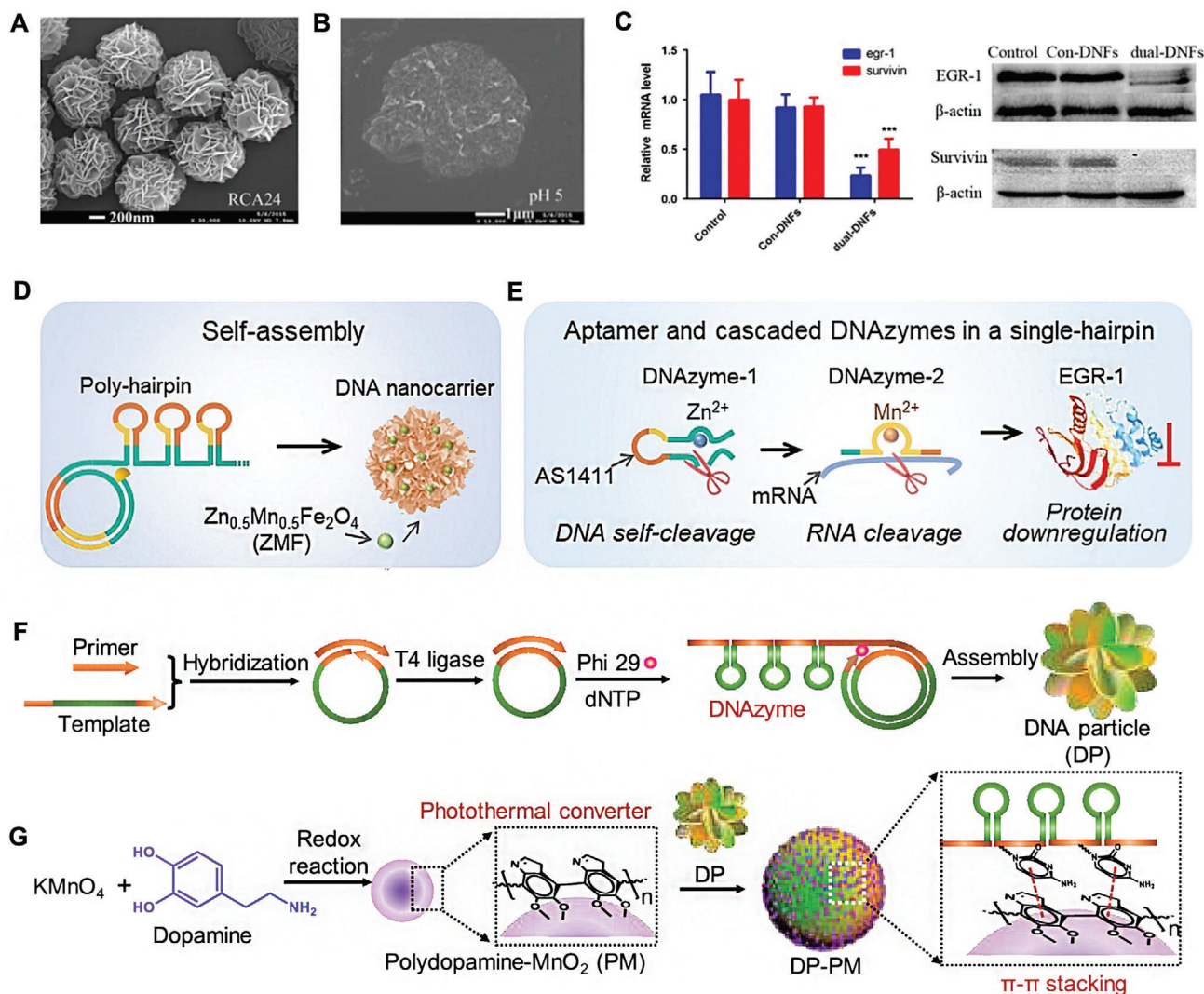
In conclusion, the combination of DNazymes with other multifunctional DNA materials can enhance DNzyme function and stability, but also endow other DNA materials with the function of mRNA depletion. This complimentary design greatly enriches the design of DNzyme-based synergistic therapies.

#### 4.2.2. DNzyme-Incorporated Structures with the RCA Strategy

RCA is a nucleic-acid-based synthesis technology for micro-/nanomaterials.<sup>[149]</sup> By designing a fixed-sequence template, isothermal amplification of the repetitive sequence can be realized with the help of RNA or DNA polymerase.<sup>[131]</sup> In addition, DNA-based metal-complex structures can be constructed. For instance, after the addition of magnesium pyrophosphate ( $Mg_2PPI$ ), dense accumulation of DNA nanoflowers (NFs) can be achieved through nucleic-acid-driven crystallization. In a recent study, programmable RCA-based structures were applied to nucleic acid drug delivery systems capable of responding to target signals.<sup>[150]</sup> Different functional sequence units were included in the template chain. For example, the prostate-specific membrane antigen (PSMA)-binding aptamer served as a cell-targeting aptamer, and cholesterol-labeled DNA binding strands could achieve a synergistic enhancing effect on cellular uptake, while an ATP-binding aptamer was applied to construct a ratiometric aptasensing switch.<sup>[150]</sup> Therefore, based on this design, DNzyme sequences can be added to the template chain to achieve repeat fragment replication for efficient catalytic treatment of diseases.<sup>[151]</sup>

Jin et al. programmed two types of DNazymes (silencing EGR-1 and survivin mRNA), together with AS1411 aptamer sequences, to build into the template chain and prepared DNA nanoflowers under the action of  $Mg_2PPI$ .<sup>[152]</sup> Under acidic conditions,  $Mg_2PPI$  decomposition generates  $Mg^{2+}$  ions, which could be used as a cofactor for the two DNazymes, increasing their ability to recognize and cleave the target mRNAs. The TEM structure of the nanoparticles is shown in Figure 7A, and the nanoflower structure was destroyed under acidic conditions (Figure 7B), enabling targeted degradation within the tumor microenvironment. At the same time, both mRNAs were significantly downregulated from a therapeutic standpoint, which showed that DNazymes could achieve improved effects even without any structural modification (Figure 7C). In another example, a multifunctional DNA nanoflower was prepared under RCA guidance, and its template strand contained an effective autophagy mRNA inhibitor, DNazymes, a binding site for the tumor-targeting aptamer, and a photosensitizer chlorin e6 (Ce6) acceptor.<sup>[153]</sup> The multifunctional DNA nanoflower could specifically target tumor cells, produce Ce6-mediated reactive oxygen species (ROS) under light, and improve DNzyme bioavailability, resulting in enhanced autophagy inhibition.

DNazymes can cleave intracellular RNA genes, as well as DNA sequences generated under RCA guidance. Self-catabolic DNA nanoflowers with high stability can be synthesized using a



**Figure 7.** Rolling-circle-amplification-mediated nanostructure construction of DNAzymes. A) TEM image of DNAzyme-based nanoflower with acidic pH-responsive ability. B) TEM image of DNAzyme nanoflower after incubation within acidic buffer. C) The gene regulation effect of DNAzyme NPs within cells. A–C) Adapted with permission.<sup>[152]</sup> Copyright 2017, the Author(s), published by Springer Nature. D) The DNAzyme/Zn<sub>0.5</sub>Mn<sub>0.5</sub>Fe<sub>2</sub>O<sub>4</sub> (ZMF) composite nanosystem fabrication. E) Self-cleavage and gene regulation of hierarchical nanomaterials. D,E) Adapted with permission.<sup>[154]</sup> Copyright 2021, Wiley-VCH. F) DNAzyme-incorporated nanoflower for photothermal converter loading. G) The fabrication of KMnO<sub>4</sub>-dopamine-based photothermal converter. F,G) Adapted with permission.<sup>[156]</sup> Copyright 2021, American Chemical Society.

RCA strategy<sup>[154]</sup> for sequential activation of DNAzyme-mediated gene silencing. These DNA nanocomposites contain two-stage space-time DNAzymes, with the functional DNA component amplified hundreds or thousands of times. From Figure 7D, we can see that DNA nanoflowers can achieve self-assembly mediated only by RCA, without the assisted growth of metal ions. Promoter-like Zn–Mn–Ferrite nanoparticles composed of multiple metal ions were loaded into the pores on the nanoflower surface. Under high GSH conditions, metal nanoparticles can be degraded to release DNAzyme cofactors, which drives DNAzyme-1 inside the nanoflowers to achieve self-structural cleavage and mediate their collapse (Figure 7E). Subsequently, second-stage DNAzymes cleave intracellular oncogenes. The simultaneous release of Zn<sup>2+</sup>, Mn<sup>2+</sup>, and Fe<sup>2+</sup> can initiate a Fenton's reaction,

resulting in high-efficiency gene/chemical kinetic therapy. The system showed remarkable tumor growth inhibition in a breast cancer mouse model, shedding new light on DNAzymes as fundamental components of responsive biomaterials. Moreover, this process significantly improved DNAzyme activity and target responses, as the nanoplatform could regulate the event order, which is an attractive way to regulate cell function and fate.

Interestingly, the catalytic activity of DNAzymes increased with increasing temperature.<sup>[155]</sup> Yang and co-workers self-assembled DNAzymes into nanoparticles using the RCA method and loaded dopamine-mediated photothermal materials onto the nanoparticle surface pores<sup>[156]</sup> (Figure 7F). Nanocomposites accumulated at the tumor site, where polydopamine induced a temperature rise through photothermal conversion (Figure 7G). In

vitro and in vivo experiments showed that the temperature increase enhanced the DNzyme mRNA cleavage activity and promoted epidermal growth receptor (GFR-1) protein downregulation in tumor cells. Hence, RCA technology has become a powerful tool to maximize the therapeutic capabilities of DNzymes with many sophisticated functional purposes.

#### 4.2.3. DNzyme-Integrated Structures Using CHA

CHA is a technology for isothermal amplification of autonomous DNA devices using hairpin structures as fuel.<sup>[157]</sup> Typically, this hairpin reaction process is based on toehold-mediated strand displacement.<sup>[158]</sup> Hairpin sequences can be opened 10–100 times faster through an external toehold than an internal toehold when designing DNA hairpin structures.<sup>[158b]</sup> CHA is often used to detect specific gene sequences.<sup>[159]</sup> Hence, the greatest advantage of CHA combined with DNzymes is sequence-responsive DNzyme self-assembly.<sup>[160]</sup> Moreover, hairpin structures can also be easily self-constructed to form nanospheres or nanowires.<sup>[161]</sup> This approach results in a higher local concentration of reaction components, and spatial limitations can be utilized to accelerate the reaction rate.<sup>[162]</sup>

For example, Wei et al. developed a therapeutic DNzyme system based on CHA design.<sup>[127a]</sup> In this system, Mn<sup>2+</sup>-based honeycomb MnO<sub>2</sub> nanosponge (hMNS) particles were fabricated and combined with a DNzyme prodrug to conduct hybridization-chain-reaction (HCR)-guided intracellular self-assembly of DNzymes.<sup>[127a]</sup> hMNS not only delivered DNzymes, but also provided Mn<sup>2+</sup> as a DNzyme cofactor and magnetic resonance imaging agent (Figure 8A). Figure 8B,C shows the assembly pattern of DNA sequences guided by CHA. First, as shown in Figure 8B, the target sequence miR-21 can stimulate independent cross-hybridization of hairpin sequences (H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, and H<sub>4</sub>) to long linear nanowires. In detail, based on the chain replacement reaction mediated by the toehold, H<sub>1</sub> was opened by the target sequence miR-21 by hybridizing with the a–b (pink) domain of H<sub>1</sub>. The c–b\* (pink) sequence in the open H<sub>1</sub> was exposed and immediately hybridized with the b–c\* (blue) sequence of hairpin H<sub>2</sub>. Then, the H<sub>2</sub> structure was opened, and the b\*–d (blue) sequence was released, complementary to the d\*–b (pink) sequence within H<sub>3</sub>. Subsequently, the opened H<sub>3</sub> included the exposed e–b\* (pink) domain, which could hybridize with the b–e\* (blue) sequence of hairpin H<sub>4</sub> to release the target sequence b\*–a\*, which can open H<sub>1</sub> again. Therefore, during HCR, miR-21 can excite the hairpins of H<sub>1</sub>, H<sub>2</sub>, H<sub>3</sub>, and H<sub>4</sub> to repeatedly cross-link as nanowires. Furthermore, DNzymes were assigned to different hairpin structures and did not become catalytically active until self-assembled. Catalyzed by miRNA-21, DNzymes assembled into DNA nanowires, and some fluorescent groups also achieved light transfer to achieve miRNA detection (Figure 8C).

In addition to single-factor constructs, multiple types of DNzymes can also be combined using CHA.<sup>[127b]</sup> For example, a self-sufficient therapeutic nanosystem was realized by finely designing a DNzyme prodrug and MnO<sub>2</sub> into biocompatible nanocapsules with tumor-specific recognition/activation.<sup>[127b]</sup> Similarly, the MnO<sub>2</sub> component immediately decomposed into Mn<sup>2+</sup> ions to supplement the indispensable DNzyme cofac-

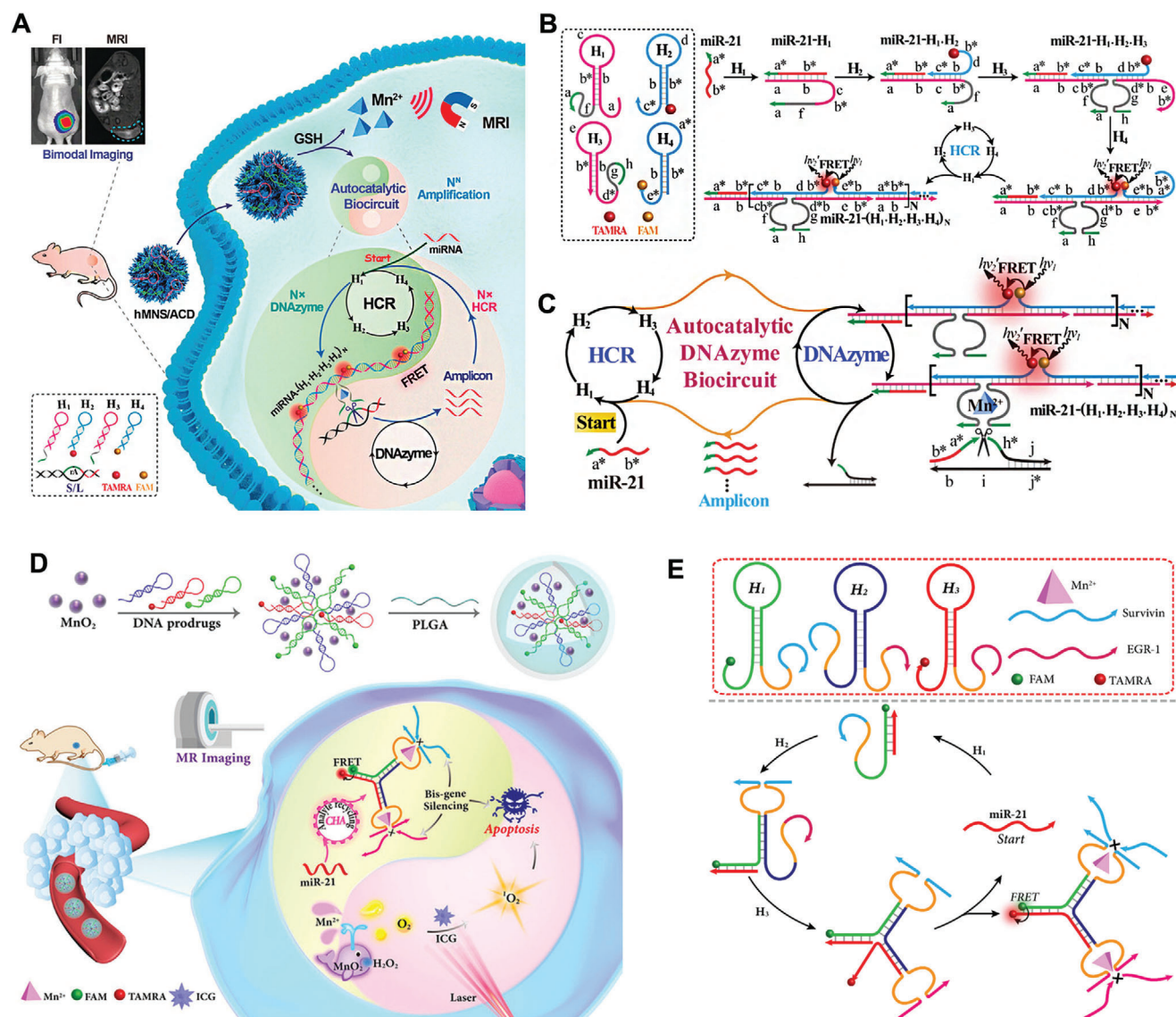
tors as needed. Subsequently, the endogenous microRNA catalyzed the amplified assembly of DNA prodrugs via CHA, simultaneously silencing two key tumor-associated mRNAs mediated by DNzymes (Figure 8D). The CHA mechanism is shown in Figure 8E. Survivin and EGR-1-targeted DNzymes were assigned to different hairpin structures to design DNzyme prodrugs. Activated by intracellular high-expression miRNAs, the hairpin structure can be opened to construct activated DNzymes. Furthermore, Liu et al. expanded the abovementioned DNzyme prodrug system into a structure with a sticky end and formed DNA nanospheres by adding adhesion chains for in situ detection of miRNA.<sup>[161]</sup> Therefore, nanosystem design based on CHA can construct DNzymes with an accurate gene sequence response. Next, we will further analyze DNzyme systems constructed based on CHA technology in combination with other treatment strategies.

#### 4.3. Nanomaterial-Based Exploration of Off-Target Effects

Gene regulation therapy with tumor/healthy tissue discrimination is an important research direction for DNzymes. Currently, DNzyme-based studies focus on the separate regulation of genes in healthy and cancerous cells. Using surfactant-assisted synthesis, Liu et al. successfully prepared GSH-responsive photosensitive ultrathin 2D nanosheets and adsorbed Ce6–DNzyme onto their surface to form a Ce6–DNzyme/[Cu(tz)] therapeutic system.<sup>[163]</sup> When the nanosystem entered the tumor tissue, the high expression of GSH decomposed the nanosheet and mediated targeted DNzyme release to cancer cells to catalyze mRNA cleavage. Simultaneously, under laser irradiation at 660 nm, the Ce6 group can generate <sup>1</sup>O<sub>2</sub> for type II photodynamic therapy (PDT), and the nanocarrier can mediate the generation of ·OH in type I PDT under laser irradiation at 808 nm. Importantly, this tumor-specific DNzyme release platform showed excellent EGR-1 cancer-cell-targeted gene silencing, with mRNA inhibited by 84% in MCF-7 (human breast cancer cells) and only 6% in MCF-10A (normal human breast epithelial cells).

In addition to nanocarriers, DNzyme modification can also achieve tumor-specific gene regulation. Wang et al. developed m6A-modified DNzymes. Intracellular fat mass and obesity-associated protein can specifically remove the m6A-caging group and promote DNzyme return to its active configuration. DNzyme activation by simple demethylase assists methylase knockout and mediates tumor cell apoptosis.<sup>[164]</sup>

Intelligent nanomachines that rely on DNzyme logic gates as the center of control have also contributed to discriminating between cancerous and healthy cells. Wang et al. constructed an intelligent nanomachine by integrating a double-responsive DNzyme logic system with a Prussian blue analog MOF (Figure 9A,B).<sup>[165]</sup> The nanomachine could regulate catalase expression by sensing miRNA-21 in cancer cells, resulting in H<sub>2</sub>O<sub>2</sub> accumulation. H<sub>2</sub>O<sub>2</sub> was also recognized by DNzymes, which further amplified their gene therapy function (Figure 9C). By contrast, this DNzyme logic gate was silent in normal cells due to the low levels of miR-21 and H<sub>2</sub>O<sub>2</sub> (Figure 9D). Therefore, nanodevices controlled by the DNzyme logic gate play an important role in the gene regulation of specific tumor tissues.



**Figure 8.** Construction of DNAzyme nanostructures based on catalytic hairpin assembly technology. A) DNAzymes bind to  $Mn^{2+}$  carriers to achieve intracellular nanowire self-assembly. B) Construction of DNA nanowires triggered by miRNA-21. C) Illustration of gene regulation mechanism and signal amplification effect. A–C) Adapted with permission.<sup>[127a]</sup> Copyright 2020, Wiley-VCH. D) DNAzyme prodrugs are activated intracellularly to achieve dual gene regulation. E) Schematic diagram of the structure preparation with multiple DNAzymes in one system. D,E) Adapted with permission.<sup>[127b]</sup> Copyright 2020, Wiley-VCH.

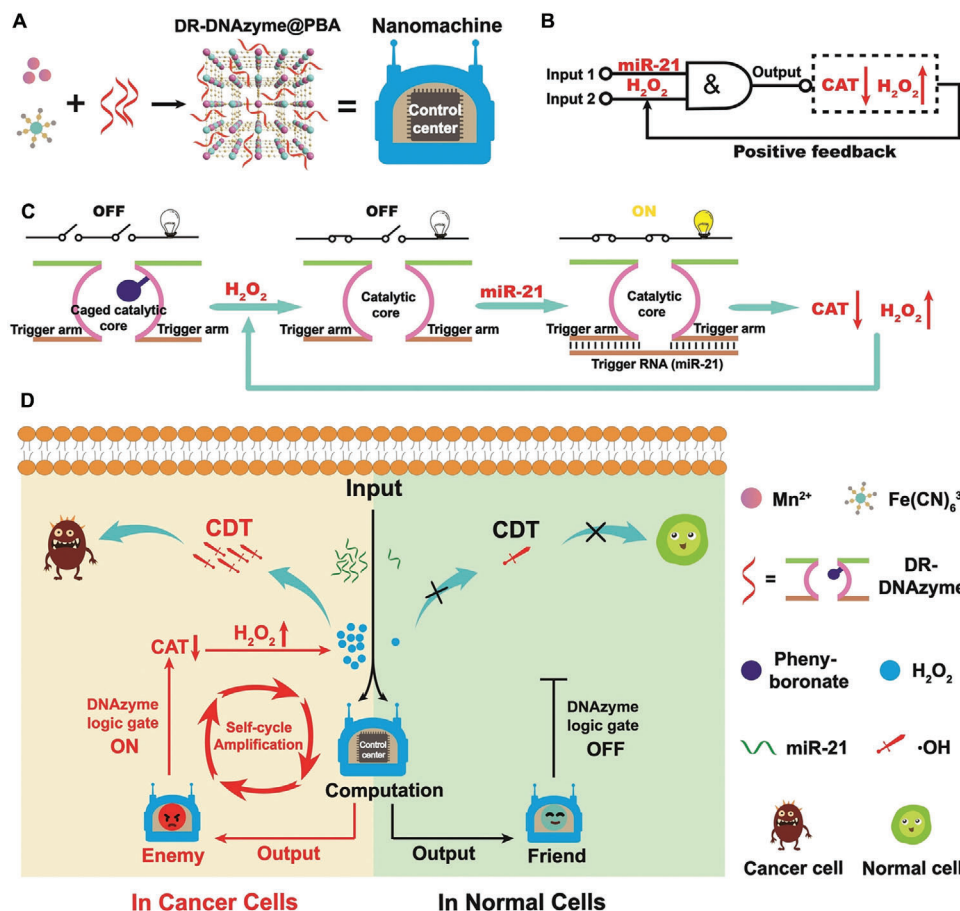
## 5. Recent Synergistic Strategies to Improve DNAzyme Therapeutic Effect

In the past five years, DNAzymes have been integrated into many aspects of gene therapy owing to the development of multiple disciplines. Single-stranded RNA, including mRNA or miRNA, can be target-silenced, and double-stranded DNA (dsDNA) can also be accurately cleaved. DNAzymes can also be applied to various biocircuit or logical gate constructions<sup>[166]</sup> used for disease-targeted therapy. The abundance of delivery vectors is essential for DNAzyme technology.<sup>[167]</sup> The carrier can supply metal cofactors for DNAzymes in specific tissues and realize multiple synergistic treatments. In addition, the types of therapeutic

DNAzymes have expanded, including 10-23, 8-17E, EtNa, and others. Here, we introduce a recently discovered DNAzyme-based synergistic therapy, with a specific focus on recent advances in different therapeutic strategies.

### 5.1. DNAzymes in Immune Therapy

Ferroptosis has been confirmed to induce immunogenic tumor cell death, thus activating the immune system.<sup>[168]</sup> In turn, the activated immune system can enhance ferroptosis by secreting interferon gamma. Therefore, the combination of ferroptosis and immunotherapy is expected to achieve complementary



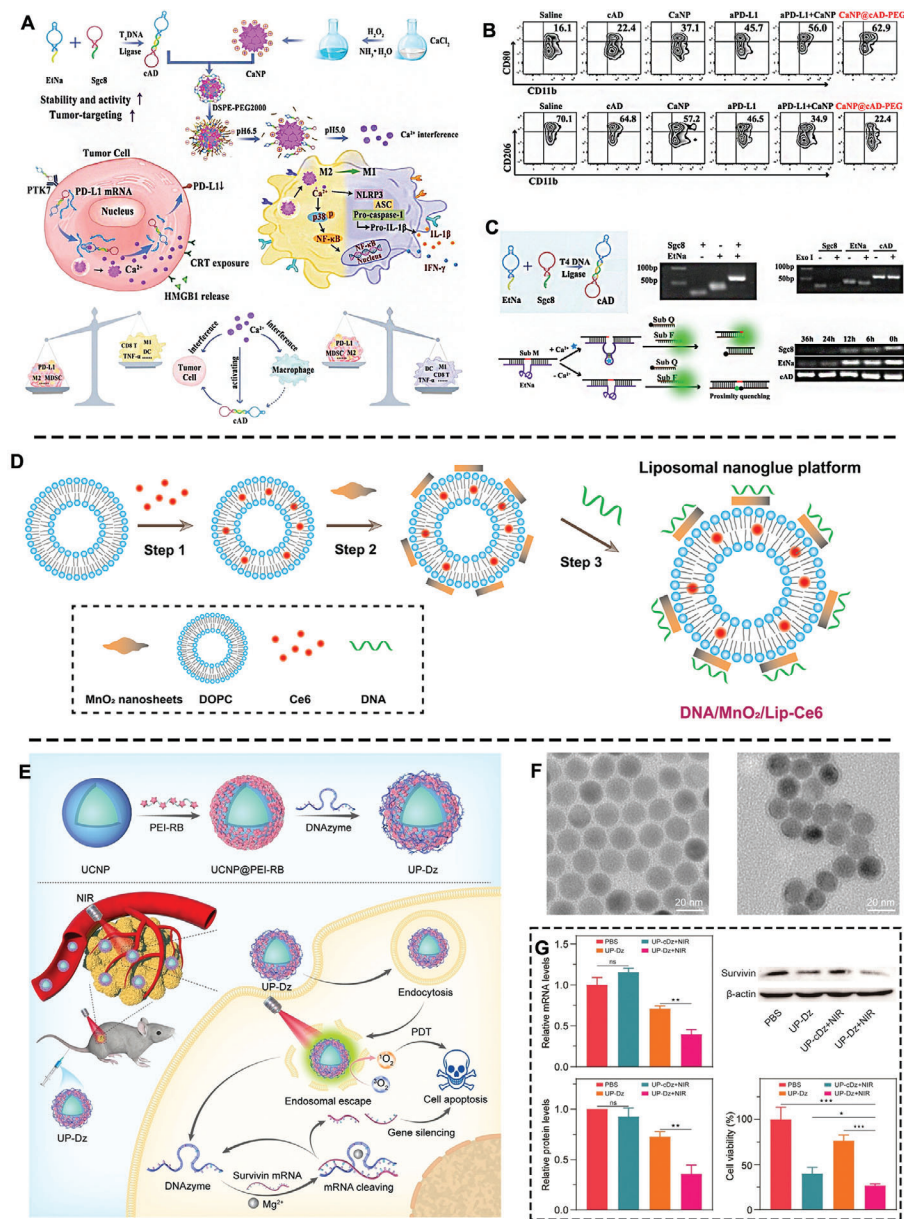
**Figure 9.** A) Design of  $\text{Mn}^{2+}$ -facilitated DNAzyme-based nanomachine. B) The logical gate for the nanomachine. C) Two input signals for triggering  $\text{H}_2\text{O}_2$  elevation and catalase (CAT) mRNA inhibition. D) The self-cycle amplification effect for targeting nanomachine within cancer cells. A–D) Adapted with permission.<sup>[165]</sup> Copyright 2022, Wiley-VCH.

advantages. However, the tumor microenvironment decreases the cancer cell response to ferroptosis, and the immune system is negatively regulated by immunosuppression pathways. Therefore, Liu et al. designed a metal–phenolic network (MPN) nanoplatform loaded with PD-L1-inhibitory DNAzyme for synergistic treatment with ferroptosis and immunosuppressive PD-1/PD-L1 pathway downregulation.<sup>[169]</sup> MPNs contained a variety of metal ions, including  $\text{Mn}^{2+}$  to activate DNAzymes and  $\text{Fe}^{3+}$  for reduction to  $\text{Fe}^{2+}$  for ferroptosis. This work provided an efficient and simple combination for tumor immune treatment.

Macrophage polarization and the PD-1/PD-L1 checkpoint within the tumor microenvironment are two key factors related to tumor immune augmentation treatment design.  $\text{Ca}^{2+}$  can regulate the polarization of tumor-associated macrophages.<sup>[146a]</sup> Therefore, combining PD-L1 mRNA-cleaving DNAzymes with calcium-ion-based materials can simultaneously regulate tumor macrophage polarization and release immunosuppression. Based on this, Shi and co-workers regulated tumor-related macrophage polarization and relieved immunosuppression using a calcium carbonate nanocarrier loaded with a DNAzyme/aptamer complex,<sup>[146a]</sup> as shown in **Figure 10A**. In this study, the Sgc8 aptamer stabilized the EtNa DNAzyme through complementary binding. Subsequently, circular

aptamer-DNAzyme (cAD) was absorbed on the surface of the  $\text{CaCO}_3$  nanoparticles for DNAzyme delivery. As a result, this system could achieve enhanced uptake through the protein tyrosine kinase 7 (PTK7) receptor, and the released  $\text{Ca}^{2+}$  could activate DNAzymes and regulate tumor-related macrophage polarization. M1 macrophages with high CD80 expression gradually increased under regulation by this nanosystem. However, the number of tumor-associated macrophages with high CD206 expression gradually decreased (Figure 10B). These results demonstrated the tumor macrophage polarization effect of the metal cofactor  $\text{Ca}^{2+}$ . Additionally, as shown in Figure 10C, the DNAzyme/aptamer complex was investigated. The cAD remained intact even when cultured with Exo I enzyme, while the single Sgc8 or EtNa units were already degraded. Furthermore, fluorescence resonance energy transfer was introduced into the design to verify DNAzyme functionality. In conclusion, combining PD-L1 mRNA-scavenging DNAzymes with calcium ions can enhance tumor immunotherapy.

In addition, recent studies have shown that DNAzymes targeting PD-L1 mRNA can be delivered in combination with other metal ions and drugs to achieve multistage immune activation therapy.<sup>[170]</sup> Therefore, anti-PD-L1 mRNA DNAzymes can be used in the field of tumor immunotherapy.



**Figure 10.** A) Combination of DNAzymes with Ca<sup>2+</sup> for immune synergistic therapy. B) Polarization regulation of tumor-related macrophages. C) Stabilization of DNAzymes/aptamer complex. A–C) Adapted with permission.<sup>[146a]</sup> Copyright 2022, Wiley-VCH. D) Fabrication of liposomal nanogluce platform for DNAzymes and photosensitizer Ce6 coloaded. Adapted with permission.<sup>[172]</sup> Copyright 2022, American Chemical Society. E) Illustration describing the DNAzyme system with enhanced lysosome escaped effect, to achieve gene–PDT combination treatment. F) TEM images of upconversion nanoparticles. G) The gene-cleavage effect for DNAzymes. E–G) Adapted with permission.<sup>[175]</sup> Copyright 2022, Wiley-VCH.

## 5.2. DNAzymes in Photothermal/Photodynamic Therapy

RNA-cleaving DNAzymes combined with photosensitizers can achieve synergistic gene/photothermal/photodynamic therapy,<sup>[171]</sup> which greatly enhances the therapeutic effect in cancer. Moreover, high temperatures can boost the catalytic efficiency of DNAzymes,<sup>[155,156]</sup> making DNAzyme-incorporated photothermal therapy a promising future opportunity. Simultaneously, integration of nanomaterials to enhance the catalytic efficiency of both photosensitizers and DNAzymes further inhibits cancer progression.<sup>[172]</sup>

Photothermal therapy (PTT) is hindered by HSP upregulation under heat stress. To date, HSP-targeted DNAzymes have been widely designed to boost photothermal therapy.<sup>[173]</sup> For instance, Zhou and co-workers developed a core–shell nanoplateform composed of a polydopamine core and a MnO<sub>2</sub> shell (PDA@MnO<sub>2</sub>). The HSP mRNA target DNAzyme was adsorbed onto the particle surface through its phosphate backbone.<sup>[174]</sup> The resulting PDA@MnO<sub>2</sub>/DNAzyme (DZ) could passively accumulate in the tumor and release Mn<sup>2+</sup> in response to intracellular GSH, which acted as a metal cofactor to activate the DNAzyme for HSP70 downregulation. As a result, the PTT efficacy of PDA@MnO<sub>2</sub>

was significantly enhanced, resulting in complete tumor elimination under laser irradiation. In a recent study, Yu et al. prepared MnO<sub>2</sub>-based liposome nanoglues that adhere to human EGR-1-targeted DNazymes (Figure 10D). The authors concluded that there are two ways to attach DNA to the nanoparticle surface: covalent and noncovalent. Because chemically modified DNA is very expensive, a noncovalent adhesive method was used in this study. In addition to DNzyme-mediated gene therapy, Ce6 loaded inside liposomes could synergistically achieve photodynamic synergistic therapy.<sup>[172]</sup> The use of liposome nanogels (DNzyme/MnO<sub>2</sub>/Lip) in combination with the gene silencing agent DNzyme and the photosensitizer Ce6 achieved effective photo-gene-therapy.

However, as described in the above chapter, the stability of DNzymes in cells is poor, which limits their clinical application. Nanocarriers are an effective solution for this issue. Using complex nanocarriers to coat DNzymes can protect them from degradation and improve their stability during in vivo transportation. However, when cells internalize nanocarriers, DNzymes still need to escape from the lysosomes to exert an effect. Zhao and co-workers developed a photocontrolled DNzyme delivery platform (Figure 10E) by combining a near-infrared (NIR)-responsive photosensitizer with upconversion nanoparticles (Figure 10F) and achieved an enhanced gene silencing effect.<sup>[175]</sup> This system allowed NIR light to produce cytotoxic reactive oxygen species, which enhanced the intracellular lysosomal escape function of the DNzyme. DNzymes were efficiently released and substrate gene knockout was achieved using laser irradiation, compared to the laser-free group (Figure 10G). Therefore, the combination of gene therapy and NIR-light-triggered photodynamic therapy is expected to solve the current problem of insufficient intracellular DNzyme activity.

Coincidentally, aggregation-induced emission (AIE) photosensitizers (thienobenzodithiophene (TBD-Br)) have also been applied to help DNzymes achieve lysosomal escape.<sup>[176]</sup> When the nanosized preparations were located inside the lysosomes of tumor cells, the <sup>1</sup>O<sub>2</sub> produced by TBD-Br under light destroyed the lysosomal structure and promoted the escape of Zn<sup>2+</sup>-coordinated DNzyme nanoparticles. In vitro and in vivo results showed that the designed AIE-based nanoplatform could induce tumor cell apoptosis through AIE photosensitizers (TBD-Br) and downregulate the EGR-1 protein to inhibit the growth of tumor cells under light irradiation.

### 5.3. DNzymes in Chemotherapy

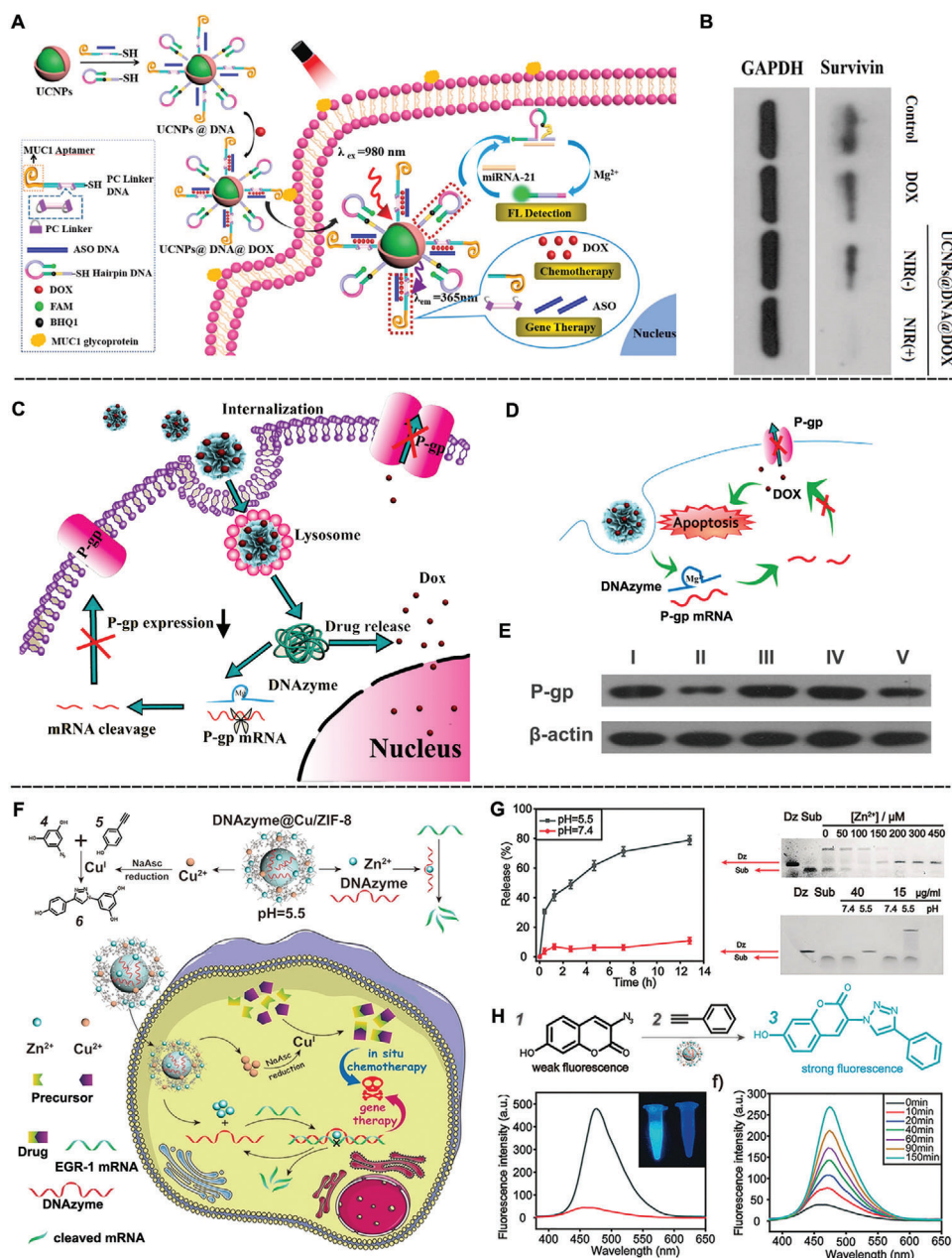
DNzyme synergy with chemotherapeutic agents is another promising therapeutic strategy.<sup>[177]</sup> Considering the design of DNzyme/chemodrug systems, the function of DNzymes can be divided into two types. The first serves to detect miRNA content during chemotherapy, and the second enhances chemotherapy effectiveness.

For example, Zhang et al. constructed a miRNA-detecting DNzyme-based theranostic platform with doxorubicin (DOX)-loaded iridium oxide as the core structure.<sup>[178]</sup> DNzymes were designed as detection probes and fixed on the surface of iridium oxide nanoparticles. In the presence of miRNA-21, DNzymes

cleaved the designed substrate and released fluorescence signals. Moreover, the nanosystem promoted photothermally controlled release of the internally loaded chemodrug DOX under NIR irradiation. Detective DNzymes can also be used in combination with upconverting nanomaterials to achieve synergistic diagnosis and treatment with chemotherapeutic drugs. Core-shell NaYF<sub>4</sub>:Yb, Tm@NaYF<sub>4</sub> upconverting nanoparticles were prepared by Zhou et al., and two functional DNA modules were riveted onto the nanoparticle surface (Figure 11A).<sup>[179]</sup> The first module consisted of FAM- and BHQ1-modified hairpin structural strands with DNzyme fragments. In the stem-ring state, FAM fluorescence was quenched by BHQ1. However, upon recognition by the oncogene miRNA-21, the stem-ring structure was opened, and the DNzyme was activated to facilitate fluorophore release. Using the advantage of multiple turnover rates, miRNA-21 could be released and start another cycle. Through this cyclic amplification process, miRNA-21 content can be sensitively detected via FAM reduction fluorescence. The second module comprised the UV-sensitive photocleavable linker-mediated DOX-releasing unit.<sup>[180]</sup> DOX could be embedded into complementary dsDNA. When upconverting nanoparticles were irradiated with 980 nm NIR light, the upconverted nanomaterials emitted 365 nm UV light<sup>[181]</sup> and cleaved the two photocleavable linkers to release ASOs and DOX for synergistic therapy. The survivin protein expression level demonstrated the NIR-mediated gene regulatory function of DNzymes (Figure 11B).

Multidrug resistance in tumors is a key reason for the low effectiveness of tumor chemotherapy. Therefore, it is particularly important to develop a therapeutic system that can reverse this phenomenon. Liang et al. prepared DNA nanoflowers with repeated DNzyme sequences for P-gp silencing (Figure 11C).<sup>[182]</sup> The system was fabricated based on RCA with the assistance of Mg<sub>2</sub>PPi. DNA nanoflowers could be loaded with DOX to achieve enhanced chemotherapy through multidrug resistance gene inhibition. Specifically, after the nanosystem entered the cells, Mg<sub>2</sub>PPi was degraded at a lower pH, which promoted lysosomal rupture and DNzyme sequence release. Then, the P-gp mRNA was silenced under Mg<sup>2+</sup> catalysis (Figure 11D). The II and V groups showed that DNzymes significantly inhibited P-gp expression (Figure 11E), which greatly enhanced chemotherapeutic efficiency (I: Control; II: P-gp-NFs; III: DOX; IV: CON-NFs-DOX; V: P-gp-NFs-DOX). In addition to P-gp, Bcl-xL is another gene that causes chemotherapy resistance. Yu et al. used DNzymes to downregulate Bcl-xL mRNA expression to enhance the radiosensitivity and chemosensitivity of the chemotherapeutic drug Fluorouracil (5-FU).<sup>[183]</sup>

Prodrug-mediated tumor in situ activation combined with DNzyme-mediated gene therapy is a new synergistic therapeutic strategy. A biMOF-encapsulated DNzyme system was developed to activate anticancer drugs in cancer cells and deplete EGR-1 mRNA mediated by DNzymes (Figure 11F).<sup>[184]</sup> Dual metals, including Cu ions, have been used in catalytic prodrug activation. Zinc ions were used to activate the DNzymes. The system minimized the side effects on normal organisms and could only kill cancer cells at the tumor site. The authors found that the DNzymes could be specifically released in an acidic release medium (Figure 11G), and the prodrug could be activated by the copper ions (Figure 11H).



**Figure 11.** DNAzymes with chemotherapy. A) Overall scheme picture for DNAzyme-and-doxorubicin-co-loaded upconverting nanoparticle system. B) The survivin expression level for different therapeutic groups. A,B) Adapted with permission.<sup>[179]</sup> Copyright 2022, Elsevier. C) Scheme picture for fabricating DNA nanoflowers (NFs) with P-gp targeting DNAzymes and DOX codelivery capacity. D) DNAzyme-mediated P-gp mRNA cleavage for enhanced chemotherapy. E) Western blot results for P-gp expression level through different treatments (I: Control; II: P-gp-NFs; III: DOX; IV: Con-NFs-DOX; V: P-gp-NFs-DOX). C–E) Adapted with permission.<sup>[182]</sup> Copyright 2022, Elsevier. F) Chemogene synergistic therapy incorporating CuI-mediated prodrug cancer cell in situ synthesis and DNAzyme-produced gene therapy. G) The pH-responsive DNAzyme release from the bimetal–organic framework, and its substrate cleavage effect. H) the prodrug synthesis during Cu<sup>I</sup>-powered click reaction. F–H) Adapted with permission.<sup>[184]</sup> Copyright 2021, Wiley-VCH.

## 6. DNAzymes in Advanced Gene Therapy

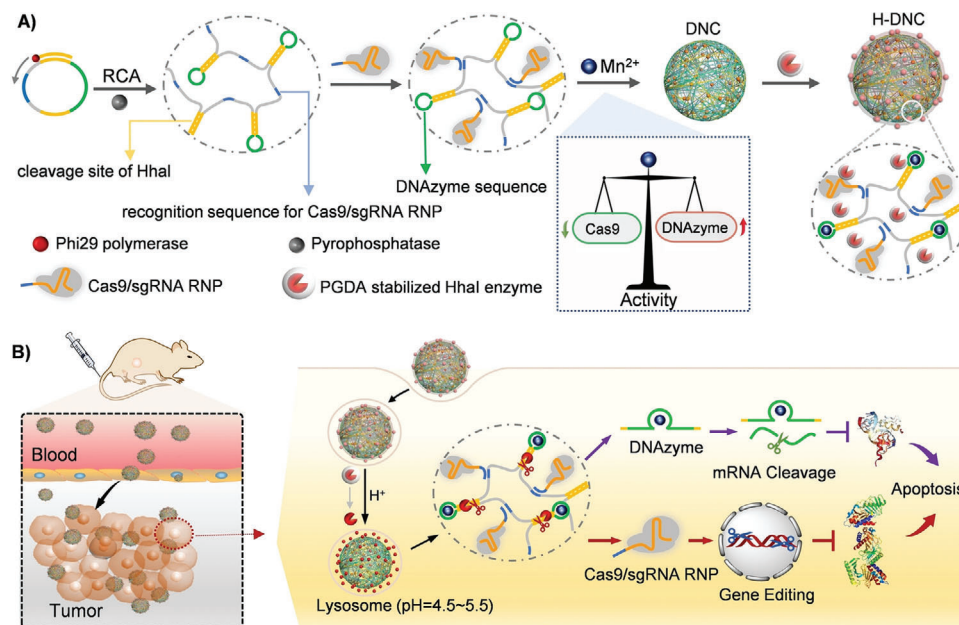
### 6.1. MiRNA-Responsive Gene Therapy

MiRNA-responsive drug delivery and drug activation systems can achieve accurately targeted disease treatment.<sup>[185]</sup> They exhibit bright development prospects for material degradation to facil-

itate therapeutic drug release<sup>[186]</sup> and for therapeutic effect of the nucleic acid materials themselves.<sup>[187]</sup>

Specifically, DNAzymes can serve as gatekeepers for payload release of stimuli.<sup>[188]</sup> In this study, the fluorescent dyes methylene blue and thionine were trapped in mesoporous silica. Mg<sup>2+</sup>- or Zn<sup>2+</sup>-dependent DNAzyme sequences riveted to the surface of the functional nanostructures, locking the dyes inside. The





**Figure 12.** Schematic of the codelivery system of Cas9/sgRNA and DNAzyme prepared by RCA method. A) Molecular design and preparation of DNA sequences. B) The gene therapy process of the nanosystem in cancer cells, including gene editing mediated by cas9/single guide RNA (sgRNA), and mRNA regulation mediated by DNAzymes. Adapted with permission.<sup>[192]</sup> Copyright 2022, Wiley-VCH.

respective DNAzymes were activated in the presence of  $Mg^{2+}$  or  $Zn^{2+}$ , resulting in specific cleavage of the respective gates and selective dye release.

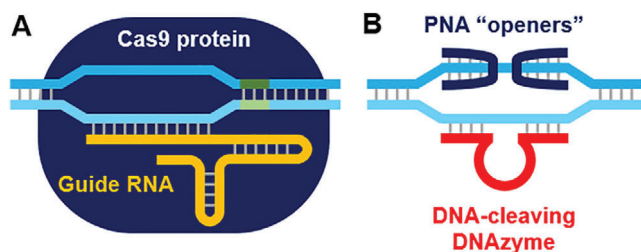
On the other hand, the abovementioned gatekeeper materials for DNAzymes can also be activated as therapeutic gene regulators. For example, a highly repetitive DNAzyme triggered by miR-21 has been reported for catalytic cleavage of EGR-1 mRNA.<sup>[189]</sup> The nanostructure could perform amplified miR-21 imaging in living cells and effectively inhibit the proliferation of cancer cells with high specificity, thereby generating sensitive diagnostic signals and accurate therapeutic effects. This work, an example of miRNA-triggered intracellular DNAzyme self-assembly, demonstrated the uniqueness and advancement of miRNA-responsive payload release. This method is superior to traditional pH-sensitive response systems, because the acidic environment within the lysosome after endocytosis is the same in healthy and cancerous cells.

MiRNA-106a has also been used to activate DNAzymes for dual gene regulation.<sup>[187]</sup> In this study, an AuNP–Ys nanoplatform was prepared by modifying AuNPs with a specifically designed Y motif, and an AuNP–Ds nanoplatform was obtained by colabeling Raman molecules with dsDNA linkers on AuNPs. When recognizing intracellular cancer-related miRNAs, the Y motif and dsDNA linker underwent miRNA-triggered ATP-driven conformational transformation, thereby significantly enhancing signal transduction. Additionally, miRNAs were released for recycling. The miRNA-106a activated system could also be used for sensitive recognition of DNase to catalyze  $Mg^{2+}$ -assisted cleavage of survivin and c-Jun mRNA, thereby performing effective double-gene silencing treatment in cancer cells.

## 6.2. CRISPR/Cas System

Clustered regularly interspaced short palindromic repeats (CRISPR/Cas) systems are an efficient and universal gene-editing platform, expected to treat diseases that traditional drugs cannot overcome. However, efficient delivery of the CRISPR/Cas system and improvements in gene editing efficiency in the tissue microenvironment are the focus of current studies.<sup>[190]</sup> In addition, the CRISPR/Cas system edits the DNA strand located in the nucleus, so it should be combined with other means to realize joint regulation of mRNA functioning in the cytoplasm. Consequently, combining CRISPR/Cas9 with DNAzymes has attracted attention recently.<sup>[191]</sup> Yang and co-workers reported a CRISPR/Cas9 nanosystem based on proton activation combined with  $Mn^{2+}$  to compress the DNA complex to form nanostructures.<sup>[192]</sup> The DNA structure in this system encoded the DNAzyme sequence and HhaI cleavage site, as well as the CRISPR/Cas9 sgRNA recognition sequence (Figure 12A). The nanostructure surface was coated with the HhaI enzyme using an acid-degradable polymer. Thus, HhaI was released from the polymer after endocytosis in response to the  $H^+$  in lysosomes. Subsequently, affected by the HhaI enzyme, nanocarriers could achieve precise disintegration and release both DNAzymes and the CRISPR/Cas9 system, which could simultaneously regulate DNA and mRNA (Figure 12B).

In addition to dual mRNA/DNA regulation, DNAzyme and the CRISPR–Cas combined systems can also enhance CRISPR–Cas application to non-nucleic-acid targets.<sup>[193]</sup> Therefore, the combination of DNAzymes and the CRISPR–Cas system has a broad research scope.



**Figure 13.** A,B) DNAzymes with the help of peptide nucleic acid for double-strand DNA regulation. A,B) Adapted with permission.<sup>[194]</sup> Copyright 2021, American Chemical Society.

### 6.3. Peptide-Nucleic-Acid (PNA)-Assisted Double-Stranded DNA Nicking “PANDA”

Although great progress has been made in DNAzyme regulation of single-stranded RNA, its effect on dsDNA has not been verified, which is ascribed to the difficulty of DNAzymes forming Watson–Crick base pairs with dsDNA. This restriction hinders the use of DNAzymes in genetic engineering. Lu and co-workers overcame this limitation, allowing DNAzymes to modify dsDNA without impacting their activity or target sequence specificity.<sup>[194]</sup> In the process of pursuing this goal, the researchers were inspired by the CRISPR/Cas system (Figure 13A) (Cas protein unlocks dsDNA and guides RNA strands to combine with complementary DNA strands) and adopted a smaller and simpler substitute PNA to achieve a similar goal. PNA is a nucleic acid analog with a strong binding affinity to dsDNA used to develop DNA manipulation tools.<sup>[195]</sup> This feature enables DNAzymes to pair with the dsDNA sequence using PNA to bind one of the double strands and leave part of the complementary single strand (Figure 13B). Using this feature, Lu and co-workers demonstrated the ability to cleave dsDNA in a highly sequence-specific manner by combining PNA with DNAzymes.

### 6.4. Cell–Cell Interaction Regulation

Regulating cell–cell interactions and cell behavior through cell surface engineering is greatly significant for biological research, including cell fate control and cell therapy. Despite many efforts to induce cell–cell assembly through various cell surface modifications triggered by macromolecules or organic metabolites, cell–cell interactions are challenging to control, including via metal-ion-triggered assembly and disassembly. Lu and co-workers reported a DNAzyme-based strategy for controllable cell–cell interactions triggered by metal ions.<sup>[196]</sup> Cleavage based on metal-dependent DNase could effectively manipulate cell behavior, including cell-to-cell connections and decomposition. During the experiments,  $Zn^{2+}$ - and  $Mg^{2+}$ -specific DNAzymes and their respective substrate chains were assembled as building blocks (Figure 13A). Then, dsDNA switches were demonstrated, which could realize two-factor-mediated decomposition (Figure 13B). In addition, the two cell spheroids could be assembled and disassembled using this method. Hence, owing to the availability of various metal-specific DNAzymes, this method can be easily applied to construct dynamic cell systems controlled by

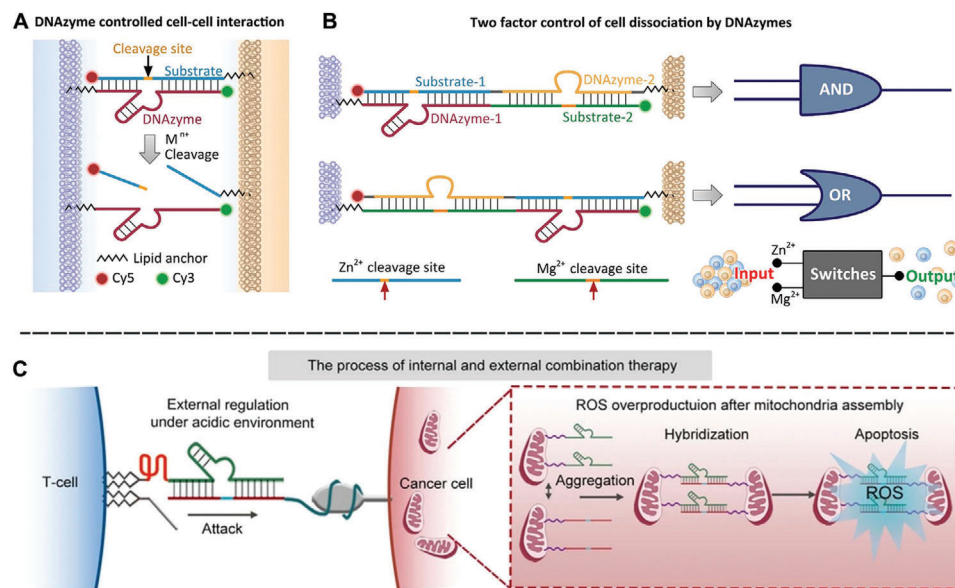
other metal ions. This technology has subsequently been used in combination with cancer therapy for dynamic intercellular and intracellular regulation using engineered DNAzyme molecular machines.<sup>[197]</sup> In this work, Lu and co-workers employed DNAzymes to achieve distance regulation between T cells/cancer cells, as well as distance regulation between mitochondria within cells. As shown in Figure 14C, the shortened distance between T cells and cancer cells is mediated by the conformational change of the G4 conjoined body under acidic conditions. Mitochondria in cells are also realized by the combination of complementary paired DNAzymes. Bound mitochondria lead to an increase in ROS, mediating apoptosis.

## 7. Other Recent Preclinical Applications for DNAzymes

In recent years, DNAzymes have mainly been used in cancer treatment. This is attributed to the vast application of emerging nanotechnologies in the field of cancer research. Few studies have been carried out in the past five years for diseases other than cancer. However, the application of DNAzymes in different research areas also provides a promising opportunity for further progression. In this section, we introduce the application of DNAzymes to develop treatment strategies in fields other than cancer.

Nine incurable polyglutamine (polyQ) diseases with neurodegenerative characteristics are mediated by repeated cytosine–adenine–guanine (CAG) amplification. Therefore, DNAzymes based on repeated CAG cleavage can improve cell viability without affecting the mitochondrial metabolism. Ashizawa and co-workers found that DNAzyme activity remained stable for at least one month after delivery into the mouse brain.<sup>[198]</sup> The DNAzymes significantly reduced the high-molecular weight Ataxia Type 3 Neurodegenerative Disorder (ATXN3) protein in the Spinocerebellar ataxia type 3 SCA3 mouse model. Therefore, DNAzymes are effective RNA-silencing molecules that may be used to treat a variety of polyQ diseases.

Allergic asthma is a serious chronic lung disease in both children and adults worldwide that can lead to significant morbidity and mortality. Many patients suffer from treatment-resistant asthma, indicating the need to develop new treatments for this disease. Rouge and co-workers have worked on DNAzyme-based allergic asthma therapy for decades.<sup>[51,199]</sup> In a recent study, peptide-cross-linked nucleic acid nanocapsules (NANs) were utilized to deliver GATA3-specific DNase to immune cells and regulate their transcriptional activity and behavior.<sup>[200]</sup> NANs were composed of a peptide cross-linked surfactant with inflammation-responsive degradability. GATA3-specific DNAzymes were combined with NAN for GATA3 mRNA silencing. DNAzymes could be effectively delivered to human peripheral blood mononuclear cells without the need for harsh transfection agents. In addition, mice treated with DNAzyme-loaded NANs during asthma induced by house dust mites exhibited less severe allergic lung inflammation (measured via pulmonary eosinophilia) than the control group (mice treated with house dust mites only). This study showed that peptide-cross-linked GATA3 DNAzyme–NANs have potential for use in human patients.



**Figure 14.** A) DNAzymes regulate cell–cell interactions. B) Two-factor disassembly control of cell assembly by DNAzyme-based DNA switches. A,B) Adapted with permission.<sup>[196]</sup> Copyright 2021, American Chemical Society. C) DNAzyme-mediated T cell/cancer cell communication, as well as mitochondrial communication. Adapted with permission.<sup>[197]</sup> Copyright 2022, Wiley-VCH.

## 8. Clinical Applications of DNAzymes

Currently, many phase I and II clinical trials are being conducted by medical institutions and Sterna Biologicals for DNAzymes as potential therapeutic agents to reverse cancer, inflammation, and respiratory diseases. At this stage, some DNAzymes have been demonstrated as safe and well-tolerated in humans, with positive therapeutic effects under specific conditions.

### 8.1. DNAzymes in Anticancer Therapy

A phase I and II clinical study (clinical trial ID: NCT01229942) of anti-EBV-encoded LMP1 Dz1 in combination with radiation therapy for NPC was conducted at the Xiangya Hospital of Central South University from 2009 to 2011.<sup>[29b]</sup> To date, only a few clinical results have been published. Forty patients with NPC were recruited for this randomized, double-blinded clinical study. Dz1 was first modified via two phosphorothioate linkages at both the 3′- and 5′-ends of its substrate-binding arms and then intratumorally injected into NPC patients undergoing radiation therapy. Compared to the control group, the tumor regression rate measured via magnetic resonance imaging significantly improved in the Dz1 group. Dz1 also affected tumor vasculature permeability and EBV copies. The results showed that Dz1 is safe and has therapeutic effects. The study also suggested that a larger population of patients should be recruited and that Dz1 blood circulation time and follow-up time should be extended to obtain more reliable study results.

A comprehensive preclinical toxicology study of Dz13 was performed at the University of New South Wales to study its safety.<sup>[22]</sup> The daily intravenous dose to the tumor-bearing mice was more than 30 times higher than the highest clinical dose, and there were no significant adverse effects on clinical blood, biochem-

istry, or organ histology in the mice. Dass and Choong found that single and repeated doses of Dz13 administered intravenously or via an intrauterine pump had no negative effects on blood cell counts or biochemical parameters in normal mice.<sup>[201]</sup> Tissue distribution experiments showed transient Dz13 accumulation in the liver, resulting in minimal hepatotoxicity, which was also observed in the interfering DNAzyme group. These preclinical results suggest that Dz13 may be well-tolerated in patients with cancer.

Another phase I clinical trial (clinical trial ID: AC-TRN12610000162011) of anti-c-Jun Dz-13 was carried out at the University of New South Wales from 2010 to 2012.<sup>[202]</sup> In this investigation, nine participants with nodular basal cell carcinoma received an intratumoral injection and were monitored for more than four weeks to evaluate the human safety and tolerability of Dz13. For stability, Dz13 was capped with an inverted thymine at the 3′-position and was entirely stabilized by a phosphodiester-linked base.<sup>[74a]</sup> Moreover, Dz13 was encapsulated in a cationic lipid carrier composed of 1,2-dioleoyl-*sn*-glycerol-3-phosphoethanolamine and 1,2-dioleoyl-3-trimethylammonium-propane. Fourteen days after injection, the treatment group underwent tumor resection, and biopsy samples were used to determine the expression of c-Jun and neoplastic markers. All patients completed the trial without drug-related adverse events or systemic Dz13 leakage. C-Jun expression in resected tumors was significantly reduced in all nine patients. In addition, Dz13 increased caspase-3, -8, and -9, as well as P53 expression levels and decreased Bcl-2 and MMP-9 protein expression. In addition, Dz13 could stimulate inflammation and immune cell infiltration at the tumor site. Notably, five patients (56%) exhibited reduced histologic tumor depth, and Dz13 was well-tolerated in humans, showing potential therapeutic effects.<sup>[203]</sup>

## 8.2. DNAzymes against Chronic Inflammatory Diseases

The World Health Organization lists chronic inflammatory diseases as the most cause of death,<sup>[204]</sup> considering type 2 inflammation as the main manifestation. For example, asthma<sup>[205]</sup> (>50%), ulcerative colitis, chronic obstructive pulmonary disease (COPD), and atopic dermatitis<sup>[206]</sup> are all related to type 2 inflammation. *GATA3* is an ideal target to treat type 2 inflammatory disease.<sup>[206b]</sup> *GATA3* downregulation reduces the expression of inflammatory factors and simultaneously antagonizes inflammatory processes.

Sterna Biologicals is currently working on the introduction of three different DNAzyme therapies for asthma (SB010), atopic dermatitis (SB011), and ulcerative colitis (SB012) into clinical trials. To date, Sterna Biologicals has successfully completed three phase IIa clinical trials and is promoting two leading projects, including advanced and intermediate clinical trials for asthma and ulcerative colitis. The inhalation liquid preparation SB010, topical emulsion SB011, and enema SB012 all use the patented hgd40 preparation component with an inverted thymidine residue at the 3'-end.<sup>[51]</sup> They intend to publish their research results in academic journals. The latest results are displayed on the official website of Sterna Biologicals.

Specifically, single (clinical trial ID: NCT001470911) or multiple doses (clinical trial ID: NCT01554319) of SB010 have promised safety for healthy subjects in phase Ia studies. SB010 was further subjected to a phase Ib randomized, double-blinded, placebo-controlled, parallel-group (per dose level) test with dose escalation to evaluate its safety, tolerance, and pharmacokinetics (clinical trial ID: NCT01577953). Twenty-four male patients (aged 18–45) with stable allergic asthma with airway hyperresponsiveness received three dosage levels (5, 10, and 20 mg) of SB010-atomized solution through a controlled respiratory system (AKITA2APIXNEB). The patients showed good tolerance to all prepared SB010 dosages.

A phase IIa proof-of-concept trial (clinical trial ID: NCT01743768), designed as a randomized, double-blinded, parallel-group, multicenter clinical trial, was conducted to evaluate the efficacy of SB010 as an inhaled *GATA3* antagonist for Th2-driven asthma.<sup>[207]</sup> Forty-three patients with mild asthma were recruited, and 39 eligible patients were assigned to the SB010 (21) and placebo (18) groups to examine the improvement in lung function following specific allergen challenge. Participants were exposed to the same concentration of each specific allergen predosing and 28 days postdosing. Topical administration of SB010 yielded statistically significant improvements in lung function in both early and late asthma responses, compared with placebo. SB010 was well-tolerated and safe. Consequently, Sterna Biologicals is currently conducting the GIANT-1 trial to further evaluate the effects of SB010-targeted *GATA3* in patients with moderate-to-severe asthma.<sup>[208]</sup> Participants receiving daily oral inhalation of SB010 were monitored for airway effects based on the proportion of expired nitric oxide. The safety, tolerability, and pharmacokinetics of hgd40 at repeated oral inhaled doses of SB010 were also evaluated.<sup>[209]</sup>

Additionally, Sterna Biologicals has established further technical and medical validation through hgd40-based programs in COPD<sup>[210]</sup> and atopic dermatitis (clinical trial ID: NCT02079688). A randomized, double-blinded, placebo-controlled, multicenter

clinical trial (clinical trial ID: DRKS00006087) was performed and registered in the German Clinical Trials Registry. The purpose of this clinical trial was to evaluate the feasibility and safety of inhaled SB010 to treat COPD sputum eosinophilia. Twenty-three patients were assigned to two groups (SB010, 9; placebo, 10). After 28 days of treatment, sputum eosinophil counts were reduced in patients who inhaled SB010, compared to those in the placebo group. This long-term study of SB010 demonstrated its safety and tolerability.

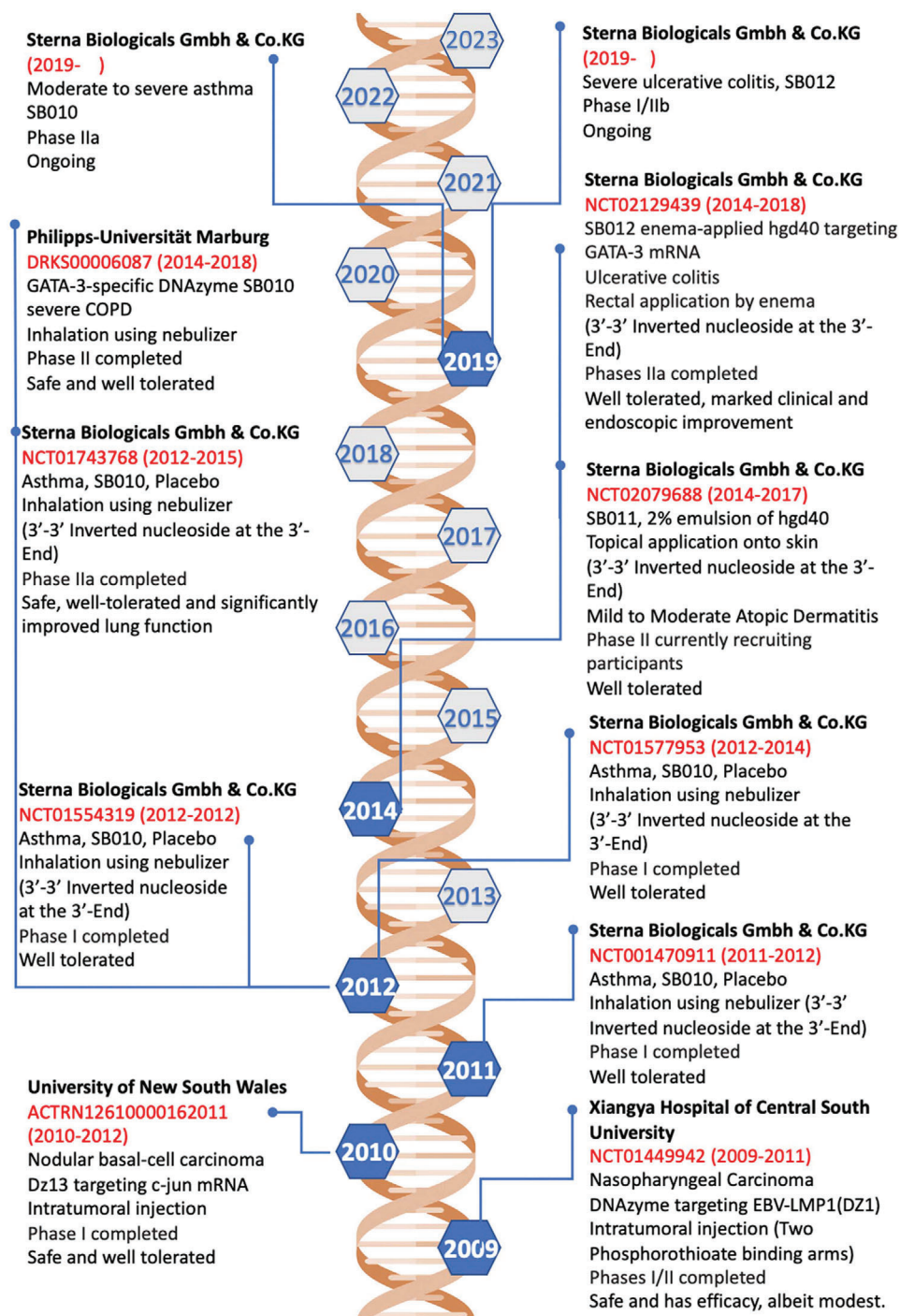
A phase IIa, single-center, randomized, vehicle-controlled, double-blinded trial was also conducted to assess the efficacy, safety, and tolerability of topical formulation SB011 applied to lesional skin in 25 patients with atopic eczema. SB011 (hgd40) was prepared as a 2% water–oil–water emulsion.<sup>[211]</sup> The results of this study have not been published yet.

An SB012 enema targeting *GATA3* mRNA was developed to treat ulcerative colitis.<sup>[212]</sup> Topical administration of SB012 to 20 patients with moderate-to-severe ulcerative colitis was well-tolerated in a prospective, multicenter, randomized, double-blinded, placebo-controlled phase IIa trial (clinical trial ID: NCT02129439). SB012 could improve clinical and endoscopic examination results. Consequently, Sterna Biologicals is currently developing a novel colon-targeted oral formulation of hgd40 for phase IIb clinical trials in patients with moderate to severe ulcerative colitis.<sup>[213]</sup>

In conclusion, treatment based on DNAzymes is in progress, and preliminary trials have shown that DNAzymes have good safety, tolerability, and efficacy, and limitless future application developments. Sterna Biologicals secured €12 000 000 and €10 000 000 in 2021 and 2022, respectively, showing that their development is on the rise. However, since the first project was established in 2009, most clinical trials have been conducted up until 2014 (Figure 15), and the number of projects has gradually decreased in recent years. Only one developer, Sterna Biologicals, was the main driving force of each clinical trial. Therefore, we hope that more companies, as well as the medical sector, will join the efforts to promote DNAzyme treatment in the future.

## 9. Conclusions and Perspectives

Therapeutic DNAzyme studies can be divided into two major periods: from early single-therapeutic application to use as biological machine components in recent years. Throughout their history, DNAzymes have demonstrated various biological applications, and research on therapeutic DNAzymes includes, but is not limited to, structural stability, disease diagnosis and treatment, delivery systems, and various advanced applications. We discussed the development model, key limitations, and current solutions for each research hotspot in the scientific community. In this review, we first elaborated on the efforts to modify the structural stability of DNAzymes in earlier studies, highlighting the existing contradictions and directions for resolution. Following the timeline, we further dissected the catalytic structural studies of DNAzymes in recent years and emphasized the concept of designing and modifying DNAzyme structures from the perspective of nucleotide function, rather than relying on large-scale screening. Subsequently, we identified various diseases treated using DNAzyme-based monotherapy, as well as the early delivery methods of DNAzymes. Subsequently, we explored



**Figure 15.** All clinical progress of DNAzymes to date.

various techniques to construct active therapeutic biomachines using DNAzymes as functional building blocks. In addition, the focus of this review was to illustrate that DNAzymes have been combined with other therapeutic and vector construction methods to achieve many types of synergistic therapeutic effects in recent years. We also provided specific introduction and analysis of the current cutting-edge applications of DNAzymes and their off-target effects. Finally, we summarized the progress of

all DNAzyme clinical trials since the first clinical project was established in 2009, focusing on the administration and structural modification methods of each DNAzyme treatment. Below, we discuss the challenges in each study area and provide insightful viewpoints.

First, although DNAzymes can effectively bind and cleave target mRNA in vitro and in vivo, their biological effects are easily influenced by many factors, such as serum stability and

catalytic efficiency. From a structural stabilization perspective, 3'-thymidine modification is commonly accepted and applied to protect DNazymes from degradation.

The length of the substrate chain and the influence of other enzymes in the solution should be fully considered when comparing the catalytic rates of DNazymes with different modified structures. In addition, several factors should be considered. First, the structure of the catalytic core should not be affected. Second, the ability of the substrate chain to dissociate from the substrate and achieve multiple turnover rates should be guaranteed. Third, the immunogenicity of the modified sequence should be avoided. The method used by Etzkorn and co-workers<sup>[109a]</sup> to predict the function of each DNzyme nucleotide using nuclear magnetic resonance and molecular simulation is a very creative design, but it requires wider scientific popularization.

Second, optimized DNzyme delivery is an excellent solution to enhance catalytic function. Recently, DNzyme vector design has been explored extensively. In contrast to early carrier designs that imitate other oligonucleotide drug carriers, new delivery systems follow the DNzyme structural and functional characteristics. For example, metal cofactors have been designed as functional carriers to deliver DNazymes. Using Mg<sup>2+</sup>- or Mn<sup>2+</sup>-mediated DNA RCA of nanoflower structures, DNazymes can be combined with other functional sequences, such as aptamers.<sup>[214]</sup> Furthermore, so far, it has been found that metals including iron, copper, and manganese can be combined with DNA sequences to form nanohybrid systems. This phenomenon provides a wide design space for the carrier design of DNazymes. Since these metal ions can not only form nanostructures with DNazymes to protect DNazymes from the influence of enzymes, but also serve as cofactors to contribute to the vitality of DNazymes. At the same time, the metal ions themselves can also achieve ion therapy. The combination of metal ions with DNazymes is very promising. Therefore, future vector design should focus on the DNzyme structural characteristics and structural advantages.

Subsequently, more diseases should be considered for therapeutic DNzyme research. Five years ago, treatments using DNazymes involved all medical fields embodied in the diversity of diseases. However, despite the enhanced catalytic ability of DNazymes, most of the investigated diseases are cancers, related to the popularity of cancer research. The authors believe that DNzyme research should not be limited to cancer, and studies on other diseases can expand the functional uses of DNazymes.

Finally, off-target DNzyme effects should be considered. In addition to targeted therapy mediated by nanocarrier systems, the DNzyme structure should be modified for target recognition. Multicomponent DNazymes (MNazymes) are an example strategy.<sup>[90,165]</sup> However, although target sequences (miRNA-155,<sup>[17]</sup> miRNA-21,<sup>[17]</sup> miRNA-10b,<sup>[215]</sup> and SARS-CoV-2<sup>[216]</sup>) have been broadly explored for biomarker sensing in ≈10 years of MNzyme development, the MNzyme substrate strand for therapeutic purposes has been mainly used for fluorescence/quencher-based signal detection, rather than target gene silencing. For example, the common stereotypical cleavage site sequence for 17E-based MNzyme is 3'-TrArGG-5'<sup>[17,90,217]</sup> and the sequence for 10-23-based MNazymes is 3'-CrUrGC-5'<sup>[7a,215,218]</sup> Hence, it is of great significance to incorporate MNazymes popular for biomarker testing into DNzyme-based target therapeutic systems. Even though DNazymes are func-

tionally similar to nucleic acid drugs such as antisense oligonucleotides and small interfering RNA (siRNA), but their unique advantages continue to attract the enthusiasm of researchers. Compared with antisense oligonucleotides, DNazymes possessed higher targeting ability. Furthermore, it has higher stability and is cheaper to synthesize than siRNA and does not rely on RNA-induced silencing complex (RISC) for silencing mechanism.<sup>[219]</sup> Therefore, we firmly believe that the research on therapeutic DNazymes will be more and more in-depth in future.

DNzyme research has only reached the tip of the iceberg. The current delay in clinical research does not mean that the research has entered a period of decline. By contrast, this has pushed scientists to identify key problems in clinical research. An increasing number of researchers are enthusiastic about DNazymes as therapeutic agents. We expect more clinical programs to be implemented and more institutions to participate in promoting the development of DNazymes as clinical therapeutic agents.

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## Conflict of Interest

The authors declare no conflict of interest.

## Keywords

clinical advances, mono/synergistic therapy, structural design, therapeutic DNazymes

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- [1] R. R. Breaker, G. F. Joyce, *Chem. Biol.* **1994**, *1*, 223.
- [2] S. Khan, B. Burciu, C. D. M. Filipe, Y. Li, K. Dellinger, T. F. Didar, *ACS Nano* **2021**, *15*, 13943.
- [3] J. Li, Y. Lu, *J. Am. Chem. Soc.* **2000**, *122*, 10466.
- [4] a) M. M. Ali, S. D. Aguirre, H. Lazim, Y. Li, *Angew. Chem., Int. Ed.* **2011**, *50*, 3751; b) Z. Shen, Z. Wu, D. Chang, W. Zhang, K. Tram, C. Lee, P. Kim, B. J. Salena, Y. Li, *Angew. Chem., Int. Ed.* **2016**, *55*, 2431; c) R. Pandey, D. Chang, M. Smieja, T. Hoare, Y. Li, L. Soleymani, *Nat. Chem.* **2021**, *13*, 895.
- [5] M. A. Abdou Mohamed, H. N. Kozlowski, J. Kim, K. Zagorovsky, M. Kantor, J. J. Feld, S. Mubareka, T. Mazzulli, W. C. W. Chan, *ACS Nano* **2021**, *15*, 9379.

- [6] H. Peng, X.-F. Li, H. Zhang, X. C. Le, *Nat. Commun.* **2017**, *8*, 14378.
- [7] a) E. Mokany, S. M. Bone, P. E. Young, T. B. Doan, A. V. Todd, *J. Am. Chem. Soc.* **2010**, *132*, 1051; b) S. Safdar, J. Lammertyn, D. Spasic, *Trends Biotechnol.* **2020**, *38*, 1343.
- [8] J. Elbaz, M. Moshe, B. Shlyahovsky, I. Willner, *Chemistry* **2009**, *15*, 3411.
- [9] a) E. M. McConnell, I. Cozma, Q. Mou, J. D. Brennan, Y. Lu, Y. Li, *Chem. Soc. Rev.* **2021**, *50*, 8954; b) M. Liu, D. Chang, Y. Li, *Acc. Chem. Res.* **2017**, *50*, 2273; c) R. J. Lake, Z. Yang, J. Zhang, Y. Lu, *Acc. Chem. Res.* **2019**, *52*, 3275.
- [10] F. S. Santiago, H. C. Lowe, M. M. Kavurma, C. N. Chesterman, A. Baker, D. G. Atkins, L. M. Khachigian, *Nat. Med.* **1999**, *5*, 1264.
- [11] S. W. Santoro, G. F. Joyce, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 4262.
- [12] Y. Jiao, Y. Shang, N. Li, B. Ding, *iScience* **2022**, *25*, 104018.
- [13] a) W. Zhou, J. Ding, J. Liu, *Theranostics* **2017**, *7*, 1010; b) A. A. Fokina, D. A. Stetsenko, J. C. François, *Expert Opin. Biol. Ther.* **2015**, *15*, 689.
- [14] A. A. Fokina, B. P. Chelobanov, M. Fujii, D. A. Stetsenko, *Expert Opin. Drug Delivery* **2017**, *14*, 1077.
- [15] a) S. Schubert, D. C. Gül, H. P. Grunert, H. Zeichhardt, V. A. Erdmann, J. Kurreck, *Nucleic Acids Res.* **2003**, *31*, 5982; b) W. Huo, X. Li, B. Wang, H. Zhang, J. Zhang, X. Yang, Y. Jin, *Biophys. Rep.* **2020**, *6*, 256.
- [16] Y. Gao, S. Zhang, C. Wu, Q. Li, Z. Shen, Y. Lu, Z.-S. Wu, *ACS Nano* **2021**, *15*, 19211.
- [17] a) X. Huang, Y. Zhang, J. Chen, L. Zhang, Y. Xu, W. Yin, Y. Shi, S.-Y. Liu, X. Zou, Z. Dai, *Anal. Chem.* **2022**, *94*, 12221; b) D. Zhu, Y. Wei, T. Sun, C. Zhang, L. Ang, S. Su, X. Mao, Q. Li, C. Fan, X. Zuo, J. Chao, L. Wang, *Anal. Chem.* **2021**, *93*, 2226; c) N. Wang, L. Song, Y. Qiu, H. Xing, J. Li, *Sens. Actuators, B* **2019**, *286*, 250.
- [18] C. Wang, M. P. O'Hagan, Z. Li, J. Zhang, X. Ma, H. Tian, I. Willner, *Chem. Soc. Rev.* **2022**, *51*, 720.
- [19] Y. Zhang, J. Tu, D. Wang, H. Zhu, S. K. Maity, X. Qu, B. Bogaert, H. Pei, H. Zhang, *Adv. Mater.* **2018**, *30*, 1703658.
- [20] W. Hou, Q. Ni, J. Wo, M. Li, K. Liu, L. Chen, Z. Hu, R. Liu, M. Hu, *Antiviral Res.* **2006**, *72*, 190.
- [21] N. Verma, S. K. Tripathi, I. Chaudhury, H. R. Das, R. H. Das, *Shock* **2010**, *33*, 493.
- [22] H. Cai, F. S. Santiago, L. Prado-Lourenco, B. Wang, M. Patrikakis, M. P. Davenport, G. J. Maghzal, R. Stocker, C. R. Parish, B. H. Chong, G. J. Lieschke, T.-W. Wong, C. N. Chesterman, D. J. Francis, F. J. Moloney, R. S. C. Barnetson, G. M. Halliday, L. M. Khachigian, *Sci. Transl. Med.* **2012**, *4*, 139ra82.
- [23] V. L. Benson, L. M. Khachigian, H. C. Lowe, *Br. J. Pharmacol.* **2008**, *154*, 741.
- [24] N. P. Mastroiannopoulos, J. B. Uney, L. A. Phylactou, *Molecules* **2010**, *15*, 5460.
- [25] N. Zhang, *Neural Regener. Res.* **2022**, *17*, 1989.
- [26] N. Singh, A. Ranjan, S. Sur, R. Chandra, V. Tandon, *J. Biosci.* **2012**, *37*, 493.
- [27] a) R. Goila, A. C. Banerjee, *FEBS Lett.* **1998**, *436*, 233; b) S. Basu, B. Sriram, R. Goila, A. C. Banerjee, *Antiviral Res.* **2000**, *46*, 125.
- [28] J. E. Wo, X. L. Wu, L. F. Zhou, H. P. Yao, L. W. Chen, R. H. Dennin, *World J. Gastroenterol.* **2005**, *11*, 3504.
- [29] a) Z. X. Lu, M. Ye, G. R. Yan, Q. Li, M. Tang, L. M. Lee, L. Q. Sun, Y. Cao, *Cancer Gene Ther.* **2005**, *12*, 647; b) Y. Cao, L. Yang, W. Jiang, X. Wang, W. Liao, G. Tan, Y. Liao, Y. Qiu, D. Feng, F. Tang, B. L. Hou, L. Zhang, J. Fu, F. He, X. Liu, W. Jiang, T. Yang, L. Q. Sun, *Mol. Ther.* **2014**, *22*, 371.
- [30] a) K. Yang, J. C. Chaput, *J. Am. Chem. Soc.* **2021**, *143*, 8957; b) W. Zhang, N. Liu, J. Zhang, *Biosens. Bioelectron.* **2022**, *201*, 113944.
- [31] T. Taube, S. Shalpour, G. J. Seifert, M. Pfau, G. Henze, K. Seeger, *Blood* **2005**, *106*, 148.
- [32] Y. Wu, L. Yu, R. McMahon, J. J. Rossi, S. J. Forman, D. S. Snyder, *Hum. Gene Ther.* **1999**, *10*, 2847.
- [33] H. Shimoyamada, T. Yazawa, H. Sato, K. Okudela, J. Ishii, M. Sakaeda, K. Kashiwagi, T. Suzuki, H. Mitsui, T. Woo, M. Tajiri, T. Ohmori, T. Ogura, M. Masuda, H. Oshiro, H. Kitamura, *Am. J. Pathol.* **2010**, *177*, 70.
- [34] R. G. Fahmy, C. R. Dass, L. Q. Sun, C. N. Chesterman, L. M. Khachigian, *Nat. Med.* **2003**, *9*, 1026.
- [35] P. Chiodelli, S. Rezzola, C. Urbinati, F. F. Signori, E. Monti, R. Ronca, M. Presta, M. Rusnati, *Oncogene* **2017**, *36*, 6531.
- [36] L. Zhang, W. J. Gasper, S. A. Stass, O. B. Ioffe, M. A. Davis, A. J. Mixson, *Cancer Res.* **2002**, *62*, 5463.
- [37] R. Abellan, R. Ventura, S. Pichini, J. A. Pascual, R. Pacifici, S. Di Carlo, A. Bacosi, J. Segura, P. Zuccaro, *Clin. Chem. Lab. Med.* **2005**, *43*, 75.
- [38] J. Brown, E. Y. Jones, B. E. Forbes, *Trends Biochem. Sci.* **2009**, *34*, 612.
- [39] M. Zhang, H. Zhao, F. Luo, S. Luo, W. Shi, *Biotechnol. Lett.* **2011**, *33*, 911.
- [40] A. K. Dilly, P. Ekambaram, Y. Guo, Y. Cai, S. C. Tucker, R. Fridman, M. Kandouz, K. V. Honn, *Int. J. Cancer* **2013**, *133*, 1784.
- [41] C. Liu, R. Cheng, L. Q. Sun, P. Tien, *Biochem. Biophys. Res. Commun.* **2001**, *284*, 1077.
- [42] L. Yang, L. Xiao, X. Ma, M. Tang, X. Weng, X. Chen, L. Sun, Y. Cao, *Cancer Biol. Ther.* **2009**, *8*, 366.
- [43] M. H. Lee, S. C. Yeung, *Cancer Biol. Ther.* **2009**, *8*, 372.
- [44] L. Yang, Z. Lu, X. Ma, Y. Cao, L. Q. Sun, *Molecules* **2010**, *15*, 6127.
- [45] L. Yang, W. Zeng, D. Li, R. Zhou, *Oncol. Rep.* **2009**, *22*, 121.
- [46] M. A. Hallett, P. Dalal, T. W. Sweatman, T. Pourmotabbed, *Nucleic Acid Ther.* **2013**, *23*, 379.
- [47] a) G. Xiang, M. D. Schuster, T. Seki, P. Witkowski, S. Eshghi, S. Itescu, *J. Am. Coll. Cardiol.* **2005**, *46*, 536; b) G. Xiang, T. Seki, M. D. Schuster, P. Witkowski, A. J. Boyle, F. See, T. P. Martens, A. Kocher, H. Sondermeijer, H. Krum, S. Itescu, *J. Biol. Chem.* **2005**, *280*, 39394.
- [48] S. Yuan, P. Carter, M. Brzelius, M. Vithayathil, S. Kar, A. M. Mason, A. Lin, S. Burgess, S. C. Larsson, *EBioMedicine* **2020**, *59*, 102956.
- [49] P. O. Iversen, G. Nicolaysen, M. Sioud, *Am. J. Physiol.: Heart Circ. Physiol.* **2001**, *281*, H2211.
- [50] a) T. Dicke, M. Wegmann, S. Sel, H. Renz, H. Garn, *J. Allergy Clin. Immunol.* **2007**, *119*, S1; b) S. Sel, M. Wegmann, T. Dicke, S. Sel, W. Henke, A. O. Yildirim, H. Renz, H. Garn, *J. Allergy Clin. Immunol.* **2008**, *121*, 910.
- [51] N. Krug, J. M. Hohlfeld, A. M. Kirsten, O. Kornmann, K. M. Beeh, D. Kappeler, S. Korn, S. Ignatenko, W. Timmer, C. Rogon, J. Zeitvogel, N. Zhang, J. Bille, U. Homburg, A. Turowska, C. Bachert, T. Werfel, R. Buhl, J. Renz, H. Garn, H. Renz, *N. Engl. J. Med.* **2015**, *372*, 1987.
- [52] G. Caramori, K. F. Chung, P. J. Barnes, *N. Engl. J. Med.* **2015**, *373*, 1176.
- [53] I. Chaudhury, S. K. Raghav, H. K. Gautam, H. R. Das, R. H. Das, *FEBS Lett.* **2006**, *580*, 2046.
- [54] Y. Isaka, H. Nakamura, M. Mizui, Y. Takabatake, M. Horio, H. Kawachi, F. Shimizu, E. Imai, M. Hori, *Kidney Int.* **2004**, *66*, 586.
- [55] B. Grimpe, S. Dong, C. Doller, K. Temple, A. T. Malouf, J. Silver, *J. Neurosci.* **2002**, *22*, 3144.
- [56] E. Hjiantonou, S. Iseki, J. B. Uney, L. A. Phylactou, *Biochem. Biophys. Res. Commun.* **2003**, *300*, 178.
- [57] C. R. Dass, P. F. Choong, L. M. Khachigian, *Mol. Cancer Ther.* **2008**, *7*, 243.
- [58] H. Fan, X. Zhang, Y. Lu, *Sci. China: Chem.* **2017**, *60*, 591.
- [59] a) A. Akbarzadeh, R. Rezaei-Sadabady, S. Davaran, S. W. Joo, N. Zarghami, Y. Hanifepour, M. Samiei, M. Kouhi, K. Nejati-Koshki, *Nanoscale Res. Lett.* **2013**, *8*, 102; b) L. van der Koog, T. B. Gandek, A. Nagelkerke, *Adv. Healthcare Mater.* **2022**, *11*, 2100639.
- [60] Y. Li, R. Bhindi, Z. J. Deng, S. W. Morton, P. T. Hammond, L. M. Khachigian, *Int. J. Cardiol.* **2013**, *168*, 3659.
- [61] X. Ke, Y.-C. Yang, S.-L. Hong, *Med. Oncol.* **2011**, *28*, 326.
- [62] a) W. B. Liechty, D. R. Kryscio, B. V. Slaughter, N. A. Peppas, *Annu. Rev. Chem. Biomol. Eng.* **2010**, *1*, 149; b) S. Zhao, C. Huang, X. Yue,

- X. Li, P. Zhou, A. Wu, C. Chen, Y. Qu, C. Zhang, *Mater. Des.* **2022**, 220, 110850.
- [63] H. Zhang, J. Guo, Y. Wang, L. Shang, R. Chai, Y. Zhao, *Adv. Funct. Mater.* **2022**, 32, 2203829.
- [64] Z. Xing, S. Gao, Y. Duan, H. Han, L. Li, Y. Yang, Q. Li, *Int. J. Nanomed.* **2015**, 10, 5715.
- [65] a) Q. Liu, Y.-J. Kim, G.-B. Im, J. Zhu, Y. Wu, Y. Liu, S. H. Bhang, *Adv. Funct. Mater.* **2021**, 31, 2008171; b) M. Liong, J. Lu, M. Kovichich, T. Xia, S. G. Ruehm, A. E. Nel, F. Tamanoi, J. I. Zink, *ACS Nano* **2008**, 2, 889.
- [66] S. Li, M. Meng Lin, M. S. Toprak, D. K. Kim, M. Muhammed, *Nano Rev.* **2010**, 1, 5214.
- [67] K. Yehl, J. P. Joshi, B. L. Greene, R. B. Dyer, R. Nahta, K. Salaita, *ACS Nano* **2012**, 6, 9150.
- [68] I. Somasuntharam, K. Yehl, S. L. Carroll, J. T. Maxwell, M. D. Martinez, P.-L. Che, M. E. Brown, K. Salaita, M. E. Davis, *Biomaterials* **2016**, 83, 12.
- [69] S.-R. Ryoo, H. Jang, K.-S. Kim, B. Lee, K. B. Kim, Y.-K. Kim, W.-S. Yeo, Y. Lee, D.-E. Kim, D.-H. Min, *Biomaterials* **2012**, 33, 2754.
- [70] a) B.-F. Yuan, Y. Xue, M. Luo, Y.-H. Hao, Z. Tan, *Int. J. Biochem. Cell Biol.* **2007**, 39, 1119; b) H. Fan, Z. Zhao, G. Yan, X. Zhang, C. Yang, H. Meng, Z. Chen, H. Liu, W. Tan, *Angew. Chem. Int. Ed.* **2015**, 54, 4801.
- [71] R. G. Fahmy, L. M. Khachigian, *Nucleic Acids Res.* **2004**, 32, 2281.
- [72] C. R. Dass, E. G. Saravolac, Y. Li, L. Q. Sun, *Antisense Nucleic Acid Drug Dev.* **2002**, 12, 289.
- [73] L.-Q. Sun, M. J. Cairns, W. L. Gerlach, C. Witherington, L. Wang, A. King, *J. Biol. Chem.* **1999**, 274, 17236.
- [74] a) Y. Li, S. Du, H. Jin, J. He, *Bioorg. Med. Chem. Lett.* **2022**, 62, 128633; b) M. Chakravarthy, M. T. Aung-Htut, B. T. Le, R. N. Veedu, *Sci. Rep.* **2017**, 7, 1613.
- [75] R. Bhindi, R. G. Fahmy, H. C. Lowe, C. N. Chesterman, C. R. Dass, M. J. Cairns, E. G. Saravolac, L.-Q. Sun, L. M. Khachigian, *Am. J. Pathol.* **2007**, 171, 1079;
- [76] A. A. Fokina, M. I. Meschaninova, T. Durfort, A. G. Venyaminova, J. C. François, *Biochemistry* **2012**, 51, 2181.
- [77] B. Vester, L. B. Lundberg, M. D. Sørensen, B. R. Babu, S. Douthwaite, J. Wengel, *J. Am. Chem. Soc.* **2002**, 124, 13682.
- [78] a) H. Unwalla, A. C. Banerjee, *Biochem. J.* **2001**, 357, 147; b) A. Abdelgany, M. Wood, D. Beeson, *J. Gene Med.* **2007**, 9, 727.
- [79] a) M. B. Appaiahgari, S. Vрати, *Mol. Ther.* **2007**, 15, 1593; b) Y. Y. Xie, X. D. Zhao, L. P. Jiang, H. L. Liu, L. J. Wang, P. Fang, K. L. Shen, Z. D. Xie, Y. P. Wu, X. Q. Yang, *Antiviral Res.* **2006**, 71, 31; c) S. K. Silverman, *Trends Biochem. Sci.* **2016**, 41, 595.
- [80] C. Wahlestedt, P. Salmi, L. Good, J. Kela, T. Johnsson, T. Hökfelt, C. Broberger, F. Porreca, J. Lai, K. Ren, M. Ossipov, A. Koshkin, N. Jakobsen, J. Skouv, H. Oerum, M. H. Jacobsen, J. Wengel, *Proc. Natl. Acad. Sci. USA* **2000**, 97, 5633.
- [81] K. Fluiter, A. L. ten Asbroek, M. B. de Wissel, M. E. Jakobs, M. Wissenbach, H. Olsson, O. Olsen, H. Oerum, F. Baas, *Nucleic Acids Res.* **2003**, 31, 953.
- [82] M. A. Guvakova, L. A. Yakubov, I. Vlodayky, J. L. Tonkinson, C. A. Stein, *J. Biol. Chem.* **1995**, 270, 2620.
- [83] V. M. Jadhav, V. Scaria, S. Maiti, *Angew. Chem., Int. Ed.* **2009**, 48, 2557.
- [84] H. Suryawanshi, M. K. Lalwani, S. Ramasamy, R. Rana, V. Scaria, S. Sivasubbu, S. Maiti, *ChemBioChem* **2012**, 13, 584.
- [85] Y. Wang, K. Nguyen, R. C. Spitale, J. C. Chaput, *Nat. Chem.* **2021**, 13, 319.
- [86] a) R. C. Spitale, J. C. Chaput, *Nat. Chem.* **2022**, 14, 859; b) K. Nguyen, Y. Wang, W. E. England, J. C. Chaput, R. C. Spitale, *J. Am. Chem. Soc.* **2021**, 143, 4519; c) A. I. Taylor, P. Holliger, *Nat. Chem.* **2022**, 14, 855.
- [87] S. T. Crooke, *Methods Enzymol.* **2000**, 313, 3.
- [88] X.-H. Zhao, L. Gong, X.-B. Zhang, B. Yang, T. Fu, R. Hu, W. Tan, R. Yu, *Anal. Chem.* **2013**, 85, 3614.
- [89] J. Victor, G. Steger, D. Riesner, *Eur. Biophys. J.* **2018**, 47, 333.
- [90] W. Zhou, Q. Chen, P. J. Huang, J. Ding, J. Liu, *Anal. Chem.* **2015**, 87, 4001.
- [91] W. Shen, R. Wang, Q. Fan, Y. Li, Y. Cheng, *Gene Ther.* **2020**, 27, 383.
- [92] G. Gao, X. Sun, G. Liang, *Adv. Funct. Mater.* **2021**, 31, 2100738.
- [93] X.-L. Ding, M.-D. Liu, Q. Cheng, W.-H. Guo, M.-T. Niu, Q.-X. Huang, X. Zeng, X.-Z. Zhang, *Biomaterials* **2022**, 281, 121369.
- [94] R. Saran, Z. Huang, J. Liu, *Coord. Chem. Rev.* **2021**, 428, 213624.
- [95] a) H. Asanuma, Y. Kamiya, H. Kashida, K. Murayama, *Chem. Commun.* **2022**, 58, 3993; b) R. Johnson, *Nat. Chem.* **2015**, 7, 94.
- [96] a) R. El-Khoury, M. J. Damha, *Acc. Chem. Res.* **2021**, 54, 2287; b) X.-J. Zhang, Z. Zhao, X. Wang, M.-H. Su, L. Ai, Y. Li, Q. Yuan, X.-Q. Wang, W. Tan, *Natl. Sci. Rev.* **2023**, 10, nwac107.
- [97] Y. Liu, Y. Wang, *Nat. Chem.* **2022**, 14, 725.
- [98] Y. Zhou, C. Zhou, *Nat. Chem.* **2021**, 13, 299.
- [99] M. Takahashi, H. Li, J. Zhou, P. Chomchan, V. Aishwarya, M. J. Damha, J. J. Rossi, *Mol. Ther.–Nucleic Acids* **2019**, 17, 615.
- [100] H. Rosenbach, J. Borggräfe, J. Victor, C. Wuebben, O. Schiemann, W. Hoyer, G. Steger, M. Etzkorn, I. Span, *Biol. Chem.* **2020**, 402, 99.
- [101] Z. Li, J. Wang, Z. Zhou, M. P. O'Hagan, I. Willner, *ACS Nano* **2022**, 16, 3625.
- [102] M. V. Sednev, A. Liaqat, C. Höbartner, *J. Am. Chem. Soc.* **2022**, 144, 2090.
- [103] a) B. Vester, L. H. Hansen, L. B. Lundberg, B. R. Babu, M. D. Sørensen, J. Wengel, S. Douthwaite, *BMC Mol. Biol.* **2006**, 7, 19; b) L. Ma, J. Liu, *iScience* **2020**, 23, 100815.
- [104] L. Robaldo, J. M. Montserrat, A. M. Iribarren, *Bioorg. Med. Chem. Lett.* **2010**, 20, 4367.
- [105] a) Q. Wang, D. Zhang, M. Cheng, J. He, K. Liu, *Acta Pharm. Sin. B* **2012**, 2, 28; b) Y. Liu, Z. Li, G. Liu, Q. Wang, W. Chen, D. Zhang, M. Cheng, Z. Zheng, K. Liu, J. He, *Chem. Commun.* **2013**, 49, 5037; c) M. H. Rätz, M. Hollenstein, *Mol. Biosyst.* **2015**, 11, 1454.
- [106] a) P. Li, S. Du, Y. Li, J. He, *Molecules* **2017**, 22, 1011; b) J. Zhu, Z. Li, Q. Wang, Y. Liu, J. He, *Bioorg. Med. Chem. Lett.* **2016**, 26, 4462; c) X. Yang, Z. Xiao, J. Zhu, Z. Li, J. He, L. Zhang, Z. Yang, *Org. Biomol. Chem.* **2016**, 14, 4032.
- [107] Y. Wang, E. Liu, C. H. Lam, D. M. Perrin, *Chem. Sci.* **2018**, 9, 1813.
- [108] a) H. Rosenbach, J. Victor, M. Etzkorn, G. Steger, D. Riesner, I. Span, *Molecules* **2020**, 25, 3100; b) J. H. Yum, H. Sugiyama, S. Park, *Chem. Rec.* **2022**, 22, 202100333.
- [109] a) J. Borggräfe, J. Victor, H. Rosenbach, A. Viegas, C. G. W. Gertzen, C. Wuebben, H. Kovacs, M. Gopalswamy, D. Riesner, G. Steger, O. Schiemann, H. Gohlke, I. Span, M. Etzkorn, *Nature* **2022**, 601, 144; b) H. Liu, X. Yu, Y. Chen, J. Zhang, B. Wu, L. Zheng, P. Haruehanroengra, R. Wang, S. Li, J. Lin, J. Li, J. Sheng, Z. Huang, J. Ma, J. Gan, *Nat. Commun.* **2017**, 8, 2006; c) A. Ponce-Salvatierra, K. Wawrzyniak-Turek, U. Steuerwald, C. Hobartner, V. Pena, *Nature* **2016**, 529, 231; d) J. Nowakowski, P. J. Shim, G. S. Prasad, C. D. Stout, G. F. Joyce, *Nat. Struct. Biol.* **1999**, 6, 151.
- [110] H. Peng, A. M. Newbigging, Z. Wang, J. Tao, W. Deng, X. C. Le, H. Zhang, *Anal. Chem.* **2018**, 90, 190.
- [111] H.-K. Kim, J. Liu, J. Li, N. Nagraj, M. Li, C. M. B. Pavot, Y. Lu, *J. Am. Chem. Soc.* **2007**, 129, 6896.
- [112] A. K. Brown, J. Li, C. M. Pavot, Y. Lu, *Biochemistry* **2003**, 42, 7152.
- [113] J. Aranda, M. Terrazas, H. Gómez, N. Villegas, M. Orozco, *Nat. Catal.* **2019**, 2, 544.
- [114] a) I. G. Gazaryan, I. P. Krasinskaya, B. S. Kristal, A. M. Brown, *J. Biol. Chem.* **2007**, 282, 24373; b) L. C. Costello, R. B. Franklin, *Pancreatic Disord. Ther.* **2013**, Suppl. 4, <https://doi.org/10.4172/2165-7092.S4-002>.



- [115] a) W. J. Moon, Y. Yang, J. Liu, *ChemBioChem* **2021**, *22*, 779; b) M. Liu, Y. Peng, Y. Nie, P. Liu, S. Hu, J. Ding, W. Zhou, *Acta Biomater.* **2020**, *110*, 242.
- [116] S. Wu, K. Zhang, Y. Liang, Y. Wei, J. An, Y. Wang, J. Yang, H. Zhang, Z. Zhang, J. Liu, J. Shi, *Adv. Sci.* **2022**, *9*, 2103534.
- [117] Q.-W. Chen, X.-H. Liu, J.-X. Fan, S.-Y. Peng, J.-W. Wang, X.-N. Wang, C. Zhang, C.-J. Liu, X.-Z. Zhang, *Adv. Funct. Mater.* **2020**, *30*, 1909806.
- [118] X. Li, Y. He, L. Yang, Z. He, J. J. Zhu, *Chem. Commun.* **2021**, 57, 6776.
- [119] Y. Li, P. Zhao, T. Gong, H. Wang, X. Jiang, H. Cheng, Y. Liu, Y. Wu, W. Bu, *Angew. Chem., Int. Ed.* **2020**, *59*, 22537.
- [120] Y. Liu, Z. Liu, R. Liu, K. Wang, H. Shi, J. Huang, *Analyst* **2021**, *146*, 3391.
- [121] a) Y. Nie, D. Li, Y. Peng, S. Wang, S. Hu, M. Liu, J. Ding, W. Zhou, *Int. J. Pharm.* **2020**, 585, 119513; b) J. T. Yi, Q. S. Pan, C. Liu, Y. L. Hu, T. T. Chen, X. Chu, *Nanoscale* **2020**, *12*, 10380.
- [122] W. Peng, S. Li, Y. Zhang, Q. Zhang, W. Li, H. Han, D. Zhou, S. Wang, J. Chang, X. Gong, *Chem. Eng. J.* **2022**, *450*, 138138.
- [123] Y. Zhao, Y. Bian, X. Xiao, B. Liu, B. Ding, Z. Cheng, P. Ma, J. Lin, *Small* **2022**, *18*, 2204047.
- [124] H. Liu, R. Jiang, Y. Lu, B. Shan, Y. Wen, M. Li, *ACS Appl. Mater. Interfaces* **2022**, *14*, 28537.
- [125] X. Meng, K. Zhang, F. Yang, W. Dai, H. Lu, H. Dong, X. Zhang, *Anal. Chem.* **2020**, *92*, 8333.
- [126] C. Liu, Y. Chen, J. Zhao, Y. Wang, Y. Shao, Z. Gu, L. Li, Y. Zhao, *Angew. Chem., Int. Ed.* **2021**, *60*, 14324.
- [127] a) J. Wei, H. Wang, Q. Wu, X. Gong, K. Ma, X. Liu, F. Wang, *Angew. Chem., Int. Ed.* **2020**, *59*, 5965; b) X. Gong, R. Li, J. Wang, J. Wei, K. Ma, X. Liu, F. Wang, *Angew. Chem., Int. Ed.* **2020**, *59*, 21648.
- [128] H. Wang, Y. Chen, H. Wang, X. Liu, X. Zhou, F. Wang, *Angew. Chem., Int. Ed.* **2019**, *58*, 7380.
- [129] J. T. Yi, T. T. Chen, J. Huo, X. Chu, *Anal. Chem.* **2017**, *89*, 12351.
- [130] J. Wang, H. Wang, H. Wang, S. He, R. Li, Z. Deng, X. Liu, F. Wang, *ACS Nano* **2019**, *13*, 5852.
- [131] J. Wang, S. Yu, Q. Wu, X. Gong, S. He, J. Shang, X. Liu, F. Wang, *Angew. Chem., Int. Ed.* **2021**, *60*, 10766.
- [132] Y. Zhao, R. Li, J. Sun, Z. Zou, F. Wang, X. Liu, *ACS Nano* **2022**, *16*, 5404.
- [133] a) X. Liu, F. Zhang, X. Jing, M. Pan, P. Liu, W. Li, B. Zhu, J. Li, H. Chen, L. Wang, J. Lin, Y. Liu, D. Zhao, H. Yan, C. Fan, *Nature* **2018**, *559*, 593; b) S. Dey, C. Fan, K. V. Gothelf, J. Li, C. Lin, L. Liu, N. Liu, M. A. D. Nijenhuis, B. Sacca, F. C. Simmel, H. Yan, P. Zhan, *Nat. Rev. Methods Primers* **2021**, *1*, 13.
- [134] S. Jiang, Z. Ge, S. Mou, H. Yan, C. Fan, *Chem* **2021**, *7*, 1156.
- [135] X. Li, F. Yang, W. Zhou, R. Yuan, Y. Xiang, *Chem. Sci.* **2020**, *11*, 8966.
- [136] a) Z. Yan, X. Shen, B. Zhou, R. Pan, B. Zhang, C. Zhao, L. Ren, J. Ming, *Sens. Actuators, B* **2021**, *326*, 128831; b) J. Xu, R. Jiang, H. He, C. Ma, Z. Tang, *TrAC, Trends Anal. Chem.* **2021**, *139*, 116257.
- [137] G. Tikhomirov, P. Petersen, L. Qian, *Nature* **2017**, *552*, 67.
- [138] a) P. Wang, M. A. Rahman, Z. Zhao, K. Weiss, C. Zhang, Z. Chen, S. J. Hurwitz, Z. G. Chen, D. M. Shin, Y. Ke, *J. Am. Chem. Soc.* **2018**, *140*, 2478; b) J. Huang, W. Ma, H. Sun, H. Wang, X. He, H. Cheng, M. Huang, Y. Lei, K. Wang, *ACS Appl. Bio Mater.* **2020**, *3*, 2779.
- [139] a) E. Torelli, M. Marini, S. Palmano, L. Piantanida, C. Polano, A. Scarpellini, M. Lazzarino, G. Firrao, *Small* **2014**, *10*, 2918; b) L. Chen, S. Luo, Z. Ge, C. Fan, Y. Yang, Q. Li, Y. Zhang, *Nano Lett.* **2022**, *22*, 1618.
- [140] J. Wang, L. Yue, Z. Li, J. Zhang, H. Tian, I. Willner, *Nat. Commun.* **2019**, *10*, 4963.
- [141] H. Ijäs, I. Hakaste, B. Shen, M. A. Kostiaainen, V. Linko, *ACS Nano* **2019**, *13*, 5959.
- [142] L. Meng, W. Ma, S. Lin, S. Shi, Y. Li, Y. Lin, *ACS Appl. Mater. Interfaces* **2019**, *11*, 6850.
- [143] X. Peng, S. Fang, B. Ji, M. Li, J. Song, L. Qiu, W. Tan, *Nano Lett.* **2021**, *21*, 6946.
- [144] L. Yu, S. Yang, Z. Liu, X. Qiu, X. Tang, S. Zhao, H. Xu, M. Gao, J. Bao, L. Zhang, D. Luo, K. Chang, M. Chen, *Mater. Today Bio* **2022**, *15*, 100276.
- [145] S. Wang, J. Ding, W. Zhou, *Analyst* **2019**, *144*, 5098.
- [146] a) J. An, M. Liu, L. Zhao, W. Lu, S. Wu, K. Zhang, J. Liu, Z. Zhang, J. Shi, *Adv. Funct. Mater.* **2022**, *32*, 2201275; b) K. Zhang, Y. Li, J. Liu, X. Yang, Y. Xu, J. Shi, W. Liu, J. Li, *CCS Chem.* **2020**, *2*, 631.
- [147] a) M. Liu, J. Fu, C. Hejesen, Y. Yang, N. W. Woodbury, K. Gothelf, Y. Liu, H. Yan, *Nat. Commun.* **2013**, *4*, 2127; b) S. D. Mason, G. A. Wang, P. Yang, Y. Li, F. Li, *ACS Nano* **2019**, *13*, 8106; c) A. J. Thubagere, W. Li, R. F. Johnson, Z. Chen, S. Doroudi, Y. L. Lee, G. Izatt, S. Wittman, N. Srinivas, D. Woods, E. Winfree, L. Qian, *Science* **2017**, *357*, eaan6558.
- [148] Y. Yin, G. Chen, L. Gong, K. Ge, W. Pan, N. Li, J. O. A. Machuki, Y. Yu, D. Geng, H. Dong, F. Gao, *Anal. Chem.* **2020**, *92*, 9247.
- [149] E. Kim, S. Agarwal, N. Kim, F. S. Hage, V. Leonardo, A. Gelmi, M. M. Stevens, *ACS Nano* **2019**, *13*, 2888.
- [150] N. Kim, E. Kim, H. Kim, M. R. Thomas, A. Najer, M. M. Stevens, *Adv. Mater.* **2021**, *33*, 2007738.
- [151] Y. Jin, H. Wang, X. Li, H. Zhu, D. Sun, X. Sun, H. Liu, Z. Zhang, L. Cao, C. Gao, H. Wang, X. J. Liang, J. Zhang, X. Yang, *ACS Appl. Mater. Interfaces* **2020**, *12*, 26832.
- [152] Y. Jin, Z. Li, H. Liu, S. Chen, F. Wang, L. Wang, N. Li, K. Ge, X. Yang, X.-J. Liang, J. Zhang, *NPG Asia Mater.* **2017**, *9*, e365.
- [153] J. Shi, D. Wang, Y. Ma, J. Liu, Y. Li, R. Reza, Z. Zhang, J. Liu, K. Zhang, *Small* **2021**, *17*, e2104722.
- [154] C. Yao, H. Qi, X. Jia, Y. Xu, Z. Tong, Z. Gu, D. Yang, *Angew. Chem., Int. Ed.* **2022**, *61*, 202113619.
- [155] Y. Li, D. Sen, *Biochemistry* **1997**, *36*, 5589.
- [156] H. Zhao, Z. Zhang, D. Zuo, L. Li, F. Li, D. Yang, *Nano Lett.* **2021**, *21*, 5377.
- [157] P. Yin, H. M. T. Choi, C. R. Calvert, N. A. Pierce, *Nature* **2008**, *451*, 318.
- [158] a) D. Y. Zhang, E. Winfree, *J. Am. Chem. Soc.* **2009**, *131*, 17303; b) S. J. Green, D. Lubrich, A. J. Turberfield, *Biophys. J.* **2006**, *91*, 2966.
- [159] X. Song, Q. Ding, J. Zhang, R. Sun, L. Yin, W. Wei, Y. Pu, S. Liu, *Anal. Chem.* **2021**, *93*, 13687.
- [160] H. Wang, Y. He, J. Wei, H. Wang, K. Ma, Y. Zhou, X. Liu, X. Zhou, F. Wang, *Angew. Chem., Int. Ed.* **2022**, *61*, 202115489.
- [161] R. Liu, S. Zhang, T.-T. Zheng, Y.-R. Chen, J.-T. Wu, Z.-S. Wu, *ACS Nano* **2020**, *14*, 9572.
- [162] Y. Wu, H.-M. Meng, J. Chen, K. Jiang, R. Yang, Y. Li, K. Zhang, L. Qu, X.-B. Zhang, Z. Li, *Chem. Commun.* **2020**, 56, 470.
- [163] S. Y. Liu, Y. Xu, H. Yang, L. Liu, M. Zhao, W. Yin, Y. T. Xu, Y. Huang, C. Tan, Z. Dai, H. Zhang, J. P. Zhang, X. M. Chen, *Adv. Mater.* **2021**, *33*, 2100849.
- [164] Q. Wang, K. Tan, H. Wang, J. Shang, Y. Wan, X. Liu, X. Weng, F. Wang, *J. Am. Chem. Soc.* **2021**, *143*, 6895.
- [165] Z. Wang, J. Yang, G. Qin, C. Zhao, J. Ren, X. Qu, *Angew. Chem., Int. Ed.* **2022**, *134*, 202204291.
- [166] Z. Li, J. Wang, I. Willner, *Adv. Funct. Mater.* **2022**, *32*, 2200799.
- [167] H. Sun, W. Ma, S. Duan, J. Huang, R. Jia, H. Cheng, B. Chen, X. He, K. Wang, *Chem. Sci.* **2021**, *12*, 12118.
- [168] H. He, L. Du, H. Guo, Y. An, L. Lu, Y. Chen, Y. Wang, H. Zhong, J. Shen, J. Wu, X. Shuai, *Small* **2020**, *16*, 2001251.
- [169] P. Liu, X. Shi, Y. Peng, J. Hu, J. Ding, W. Zhou, *Adv. Healthcare Mater.* **2022**, *11*, 2102315.
- [170] S. Du, C. Chen, S. Qu, H. Song, J. Yang, Y. Li, K. Liu, Q. Lu, W. Luo, R. Wang, X. Guan, Y. Song, X. Han, *Small* **2022**, *18*, 2203942.
- [171] D. Zhang, Y. Zheng, Z. Lin, S. Lan, X. Zhang, A. Zheng, J. Li, G. Liu, H. Yang, X. Liu, J. Liu, *Small* **2019**, *15*, 1902636.

- [172] Y. Yu, Z. Wang, S. Wu, C. Zhu, X. Meng, C. Li, S. Cheng, W. Tao, F. Wang, *ACS Appl. Mater. Interfaces* **2022**, *14*, 25126.
- [173] Y. Cao, Q. Ren, R. Hao, Z. Sun, *Mater. Des.* **2022**, *214*, 110391.
- [174] Y. Xi, X. Xie, Y. Peng, P. Liu, J. Ding, W. Zhou, *Nanoscale* **2021**, *13*, 5125.
- [175] Y. Chen, R. Zhao, L. Li, Y. Zhao, *Angew. Chem., Int. Ed.* **2022**, *61*, 202206485.
- [176] L. Shi, W. Wu, Y. Duan, L. Xu, S. Li, X. Gao, B. Liu, *ACS Nano* **2021**, *15*, 1841.
- [177] P. Liu, X. Liu, Y. Cheng, S. Zhong, X. Shi, S. Wang, M. Liu, J. Ding, W. Zhou, *ACS Appl. Mater. Interfaces* **2020**, *12*, 53654.
- [178] H. Zhang, L. Zhang, H. Zhong, S. Niu, C. Ding, S. Lv, *Chem. Eng. J.* **2022**, *430*, 132675.
- [179] Y. Zhou, W. Ma, R. Sun, B. Liu, X. Zhang, H. Yang, *Biosens. Bioelectron.* **2022**, *214*, 114549.
- [180] L. Qiu, C. Wu, M. You, D. Han, T. Chen, G. Zhu, J. Jiang, R. Yu, W. Tan, *J. Am. Chem. Soc.* **2013**, *135*, 12952.
- [181] M. Zhang, N. Wang, Z. Li, *TrAC, Trends. Anal. Chem.* **2022**, *151*, 116602.
- [182] L. Liang, W. Huo, B. Wang, L. Cao, H. Huo, Y. Liu, Y. Jin, X. Yang, *J. Colloid Interface Sci.* **2022**, *608*, 2985.
- [183] Z. Yu, J. Guo, T. Meng, L. Ge, L. Liu, H. Wang, X. Yang, *BMC Pharmacol. Toxicol.* **2022**, *23*, 13.
- [184] Z. Wang, J. Niu, C. Zhao, X. Wang, J. Ren, X. Qu, *Angew. Chem., Int. Ed.* **2021**, *60*, 12431.
- [185] M. He, M. He, C. Nie, J. Yi, J. Zhang, T. Chen, X. Chu, *ACS Appl. Mater. Interfaces* **2021**, *13*, 8015.
- [186] J. Yan, H. Zou, W. Zhou, X. Yuan, Z. Li, X. Ma, C. Liu, Y. Wang, J. M. Rosenholm, W. Cui, X. Qu, H. Zhang, *Biomater. Sci.* **2022**, *10*, 4119.
- [187] C. Dong, J. Xiong, J. Ni, X. Fang, J. Zhang, D. Zhu, L. Weng, Y. Zhang, C. Song, L. Wang, *Anal. Chem.* **2022**, *94*, 9336.
- [188] Z. Zhang, D. Balogh, F. Wang, I. Willner, *J. Am. Chem. Soc.* **2013**, *135*, 1934.
- [189] K. Quan, J. Wang, J. Li, K. Wang, J. Huang, *CCS Chem.* **2021**, *4*, 3505.
- [190] X. Xu, C. Liu, Y. Wang, O. Koivisto, J. Zhou, Y. Shu, H. Zhang, *Adv. Drug Delivery Rev.* **2021**, *176*, 113891.
- [191] X. Zhu, M.-M. Lv, J.-W. Liu, R.-Q. Yu, J.-H. Jiang, *Chem. Commun.* **2019**, *55*, 6511.
- [192] F. Li, N. Song, Y. Dong, S. Li, L. Li, Y. Liu, Z. Li, D. Yang, *Angew. Chem., Int. Ed.* **2022**, *61*, 202116569.
- [193] a) J. S. Gootenberg, O. O. Abudayyeh, J. W. Lee, P. Essletzbichler, A. J. Dy, J. Joung, V. Verdine, N. Donghia, N. M. Daringer, C. A. Freije, C. Myhrvold, R. P. Bhattacharyya, J. Livny, A. Regev, E. V. Koonin, D. T. Hung, P. C. Sabeti, J. J. Collins, F. Zhang, *Science* **2017**, *356*, 438; b) J. S. Gootenberg, O. O. Abudayyeh, M. J. Kellner, J. Joung, J. J. Collins, F. Zhang, *Science* **2018**, *360*, 439; c) Y. Xiong, J. Zhang, Z. Yang, Q. Mou, Y. Ma, Y. Xiong, Y. Lu, *J. Am. Chem. Soc.* **2020**, *142*, 207.
- [194] M. Lyu, L. Kong, Z. Yang, Y. Wu, C. E. McGhee, Y. Lu, *J. Am. Chem. Soc.* **2021**, *143*, 9724.
- [195] R. Bahal, N. A. McNeer, E. Quijano, Y. Liu, P. Sulkowski, A. Turchick, Y.-C. Lu, D. C. Bhunia, A. Manna, D. L. Greiner, M. A. Brehm, C. J. Cheng, F. López-Giráldez, A. Ricciardi, J. Beloor, D. S. Krause, P. Kumar, P. G. Gallagher, D. T. Braddock, W. M. Saltzman, D. H. Ly, P. M. Glazer, *Nat. Commun.* **2016**, *7*, 13304.
- [196] R. C. Qian, Z. R. Zhou, W. Guo, Y. Wu, Z. Yang, Y. Lu, *J. Am. Chem. Soc.* **2021**, *143*, 5737.
- [197] R.-C. Qian, Z.-R. Zhou, Y. Wu, Z. Yang, W. Guo, D.-W. Li, Y. Lu, *Angew. Chem., Int. Ed.* **2022**, *134*, 202210935.
- [198] N. Zhang, B. Bewick, J. Schultz, A. Tiwari, R. Krencik, A. Zhang, K. Adachi, G. Xia, K. Yun, P. Sarkar, T. Ashizawa, *Neurotherapeutics* **2021**, *18*, 1710.
- [199] A. K. Hartmann, D. F. Cairns-Gibson, J. J. Santiana, M. Q. Tolentino, H. M. Barber, J. L. Rouge, *ChemBioChem* **2018**, *19*, 1734.
- [200] T. D. Gavitt, A. K. Hartmann, S. S. Sawant, A. B. Mara, S. M. Szczepanek, J. L. Rouge, *ACS Nano* **2021**, *15*, 11192.
- [201] C. R. Dass, P. F. Choong, *Oligonucleotides* **2010**, *20*, 51.
- [202] G. Grassi, M. Grassi, *Lancet* **2013**, *381*, 1797.
- [203] E.-A. Cho, F. J. Moloney, H. Cai, A. Au-Yeung, C. China, R. A. Scolyer, B. Yusufi, M. J. Raftery, J. Z. Deng, S. W. Morton, P. T. Hammond, H.-T. Arkenau, D. L. Damian, D. J. Francis, C. N. Chesterman, R. S. C. Barnetson, G. M. Halliday, L. M. Khachigian, *Lancet* **2013**, *381*, 1835.
- [204] a) D. Furman, J. Campisi, E. Verdin, P. Carrera-Bastos, S. Targ, C. Franceschi, L. Ferrucci, D. W. Gilroy, A. Fasano, G. W. Miller, A. H. Miller, A. Mantovani, C. M. Weyand, N. Barzilay, J. J. Goronzy, T. A. Rando, R. B. Effros, A. Lucia, N. Kleinstreuer, G. M. Slavich, *Nat. Med.* **2019**, *25*, 1822; b) R. Pahwa, A. Goyal, I. Jialal, in *StatPearls*, StatPearls Publishing LLC, St. Petersburg, FL, USA **2022**.
- [205] J. V. Fahy, *Nat. Rev. Immunol.* **2015**, *15*, 57.
- [206] a) E.-B. Haddad, S. L. Cyr, K. Arima, R. A. McDonald, N. A. Levit, F. O. Nestle, *Dermatol. Ther.* **2022**, *12*, 1501; b) H. Garn, H. Renz, *Eur. J. Immunol.* **2017**, *47*, 22.
- [207] U. Homburg, A. Turowska, J. Kuhlmann, A. Müller, J. Renz, J. Bille, H. Renz, H. Garn, *Eur. Respir. J.* **2013**, *42*, 4858.
- [208] K. Brigitte, A. Katja, "VisionHealth's Kata Clinical app to support sterna biologicals' clinical Phase IIa asthma trial," <https://visionhealth.gmbh/en/kata-clinical-supports-sterna-biologicals-phase-ii-a-study/> (accessed: November 2021).
- [209] H. Anne, "Sterna Biologicals announces dosing of first patient in phase IIA proof-of-concept study with SB010 in moderate to severe asthma," <https://www.sterna-biologicals.com/company-news/34-sterna-biologicals-announces-dosing-of-first-patient-in-phase-ii-a-proof-of-concept-study-with-sb010-in-moderate-to-severe-asthma> (accessed: December 2021).
- [210] N. Krug, J. M. Hohlfeld, R. Buhl, J. Renz, H. Garn, H. Renz, *J. Allergy Clin. Immunol.* **2017**, *140*, 625.
- [211] a) T. Schmidts, D. Dobler, P. Schlupp, C. Nissing, H. Garn, F. Runkel, *Int. J. Pharm.* **2010**, *398*, 107; b) T. Schmidts, D. Dobler, S. von den Hoff, P. Schlupp, H. Garn, F. Runkel, *Int. J. Pharm.* **2011**, *410*, 75.
- [212] V. Popp, K. Gerlach, S. Mott, A. Turowska, H. Garn, R. Atreya, H. A. Lehr, I. C. Ho, H. Renz, B. Weigmann, M. F. Neurath, *Gastroenterology* **2017**, *152*, 176.
- [213] H. Anne, "Sterna Biologicals raise further EUR 10.0 million (≈USD 11.9 million) in series A – 2nd closing private placement," <https://www.sterna-biologicals.com/company-news/32-sterna-biologicals-raises-further-eur-10-0-million-approx-usd-11-9-million-in-series-a-2nd-closing-private-placement> (accessed: January 2021).
- [214] a) H. Zhao, J. Lv, F. Li, Z. Zhang, C. Zhang, Z. Gu, D. Yang, *Biomaterials* **2021**, *268*, 120591; b) Y. R. Baker, J. Chen, J. Brown, A. H. El-Sagheer, P. Wiseman, E. Johnson, P. Goddard, T. Brown, *Nucleic Acids Res.* **2018**, *46*, 7495.
- [215] X. Yin, B. Chen, M. He, B. Hu, *Anal. Chem.* **2021**, *93*, 4952.
- [216] H. N. Kozłowski, M. A. Abdou Mohamed, J. Kim, N. G. Bell, K. Zagorovsky, S. Mubareka, W. C. W. Chan, *Small Struct.* **2021**, *2*, 2100034.
- [217] W. Zhou, Y. Zhang, J. Ding, J. Liu, *ACS Sens.* **2016**, *1*, 600.
- [218] S. Safdar, K. Ven, J. van Lent, B. Pavie, I. Rutten, A. Dillen, S. Munck, J. Lammertyn, D. Spasic, *Biosens. Bioelectron.* **2020**, *152*, 112017.
- [219] H. K. Karnati, R. S. Yalagala, R. Undi, S. R. Pasupuleti, R. K. Gutti, *Tumor Biol.* **2014**, *35*, 9505.



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