Journal of Zhejiang University-SCIENCE B 2026 27(7):698-714
www.jzus.zju.edu.cn; www.springer.com/journal/11585
E-mail: jzus_b@zju.edu.cn

Review

https://doi.org/10.1631/jzus.B2500764

Check for updates

Programmable DNAzyme nanocatalysts for tumor immunometabolic modulation

Rongping LUO[1*], Zhuojia TANG[2*], Mengqi DING[3], Lingxiu ZOU[4], Xiaojing LIU[1], Fan YIN[5], Jianxin LYU[6], Lu WANG[2,6✉]

[1]*School of Basic Medical Sciences and Forensic Medicine, Hangzhou Medical College, Hangzhou 311399, China*
[2]*School of Laboratory Medicine and Bioengineering, Hangzhou Medical College, Hangzhou 311399, China*
[3]*Key Laboratory of Laboratory Medicine, Ministry of Education, Zhejiang Provincial Key Laboratory of Medical Genetics, School of Laboratory Medicine and Life Sciences, Wenzhou Medical University, Wenzhou 325035, China*
[4]*School of Public Health, Hangzhou Medical College, Hangzhou 311399, China*
[5]*Zhejiang University-University of Edinburgh Institute, Haining 314400, China*
[6]*Center of Clinical Laboratory, Zhejiang Provincial People's Hospital, Affiliated People's Hospital of Hangzhou Medical College, Hangzhou 310014, China*

Abstract: Programmable RNA-cleaving DNAzymes (RCDs) represent a unique class of catalytic nucleic acids that couple molecular recognition with enzyme-like activity. While DNAzymes have traditionally been explored for targeted gene regulation, recent advances in nanotechnology have repositioned them as programmable biosensing modules with stimuli-responsive therapeutic potential. When integrated into metal-oxide scaffolds, DNA-framework architectures, or metal-organic frameworks, DNAzymes form hybrid platforms that create confined catalytic microenvironments, provide enriched cofactor availability, and facilitate microenvironment-responsive activation. These engineered systems can function as nanoscale biosensing modules that respond to pH, redox gradients, metal ions, or microRNA signatures and convert these biological cues into catalytic outputs. Beyond enhancing analytical performance, such platforms may also reshape tumor immunometabolism. Through the selective cleavage of metabolic or immune-regulatory transcripts, DNAzyme nanocatalysts can directly reprogram glycolysis, redox balance, oxygen tension, and mitochondrial activity, and these metabolic changes in turn alleviate immunosuppression and promote innate and adaptive immune activation. This review outlines the mechanistic foundations of DNAzyme catalysis, summarizes recent nanoengineering strategies that endow DNAzymes with programmable sensing and stimuli-responsive functions, and discusses how these systems bridge biosensing and catalytic immunometabolic functions. We conclude with perspectives on translational challenges and opportunities, endorsing programmable DNAzyme nanocatalysts as emerging preclinical platforms for biosensing-guided immunometabolic intervention.

Key words: RNA-cleaving DNAzyme; DNAzyme nanocatalyst; Programmable biosensing; Tumor immunometabolism; Stimuli-responsive nanoplatform

1 Introduction

Tumor immunometabolism refers to the intricate interplay between cellular metabolic pathways and immune regulation within the tumor microenvironment (TME) (Kaymak et al., 2021; Kao et al., 2022; Su et al., 2024). Specifically, cancer cells undergo profound metabolic reprogramming, favoring aerobic glycolysis, glutaminolysis, and dysregulated lipid and nucleotide turnover, in order to sustain rapid proliferation and survival under hypoxia and nutrient stress (Tan et al., 2022; Huang et al., 2025). These metabolic shifts acidify the TME, increase oxidative stress, and intensify nutrient competition, thereby suppressing antitumor immunity and impairing effector T-cell and dendritic cell function. Under such metabolically imbalanced conditions, effector T cells and dendritic cells experience mitochondrial exhaustion, redox collapse, and reduced

✉ Lu WANG, wanglu622@hmc.edu.cn
* The two authors contributed equally to this work
Lu WANG, https://orcid.org/0000-0002-4614-4427
Rongping LUO, https://orcid.org/0009-0008-9241-6391
Zhuojia TANG, https://orcid.org/0009-0003-4626-1249

Received Nov. 24, 2025; Revision accepted Apr. 24, 2026; Crosschecked July 13, 2026; Published online July 21, 2026

© Zhejiang University Press 2026

bioenergetic flexibility, while regulatory T cells (Tregs) and tumor-associated macrophages (TAMs) exploit alternative carbon sources such as lactate and fatty acids to maintain immunosuppressive phenotypes (Norian et al., 2009; Scharping et al., 2021). This reciprocal crosstalk between tumor metabolism and immune dysfunction constitutes an immunometabolic barrier that restricts the efficacy of many anticancer therapies (Zheng et al., 2025). Consequently, restoring metabolic homeostasis in the TME, such as by improving mitochondrial function, rebalancing oxidative and reductive signaling, and relieving nutrient competition, has emerged as a critical strategy for promoting immune reactivation and durable tumor control.

Although metabolic intervention has emerged as a promising approach, existing strategies generally lack the selectivity and responsiveness required to operate in the heterogeneous TME. Systemic antioxidants, metabolic inhibitors, and gene-editing tools operate with limited spatial and conditional control and may not respond efficiently to fluctuating biochemical conditions (Li F et al., 2021; Wang et al., 2024). These limitations underscore the need for programmable molecular systems capable of sensing endogenous signals, distinguishing local metabolic states, and producing confined functional outputs. In this context, stimuli-responsive biosensing principles offer a useful conceptual framework for designing therapeutic systems that couple molecular recognition with localized action (Wang et al., 2022; Li et al., 2024; Liu SW et al., 2025).

RNA-cleaving DNAzymes (RCDs) offer attractive features for immunometabolic modulation, including nucleic acid programmability, metal-ion-dependent catalysis, chemical stability, and generally low intrinsic immunogenicity (Jouha and Xiong, 2021). RCDs are the most extensively studied and biomedically relevant among the various DNAzyme classes: they catalyze sequence-specific cleavage of RNA phosphodiester bonds through metal-ion-assisted transesterification (Liu et al., 2017; Wang L et al., 2023). A canonical RCD comprises two substrate-recognition arms flanking a compact catalytic core, most notably the 8-17 and 10-23 motifs, which adopt defined secondary and tertiary structures to form an active site. Their catalytic activity relies on divalent or transition-metal cofactors, which coordinate with phosphate oxygens to stabilize transition states and promote cleavage. Structural and biochemical studies have shown that these small DNA catalysts can form metal-binding geometries and base-pairing networks, enabling catalytic efficiencies comparable in some contexts to those of natural ribozymes (Khan et al., 2021). Owing to their substrate specificity, controllable activity, and excellent chemical stability, RCDs have been widely explored for gene silencing, biosensing, and intracellular regulation.

Recent advances in nanotechnology have enabled the integration of these catalytic motifs into nanoscale scaffolds, such as zinc oxide (ZnO) nanoparticles (Wang J et al., 2019; Li et al., 2022; Chen et al., 2024), DNA tetrahedra (TDNs) (Yu et al., 2022; Zhao S et al., 2025), lipid vesicles (Cai et al., 2022; Wang R et al., 2023), and metal-organic frameworks (MOFs) (Wang HM et al., 2019; Shi et al., 2020; Wang Z et al., 2022). These hybrid architectures enhance biostability, bioavailability and stimuli-responsive activation, thereby expanding DNAzymes from free catalytic oligonucleotides into multifunctional nanoplatforms capable of modulating redox balance, mitochondrial function, and metabolic signaling within the TME. Through such programmable catalytic control, DNAzyme nanocatalysts provide a versatile preclinical platform for immunometabolic intervention.

This paper provides an integrated overview of the emerging field of programmable DNAzyme nanocatalysts for tumor immunometabolic modulation. Building upon current understanding of the metabolic-immune crosstalk that shapes tumor progression, we delineate how DNAzyme-based nanostructures may enable the targeted reprogramming of dysregulated metabolic and immune states. First, we summarize the catalytic mechanisms and engineering strategies underlying DNAzyme design, emphasizing recent advances in metal-ion coordination, chemical modification, and structural programmability that contribute to selective activation and environmental responsiveness. Then we discuss representative nanoplatform architectures, including inorganic, polymeric, and DNA-framework scaffolds, that support controlled catalysis and conditionally biased activation in biological settings. Finally, we explore how DNAzyme-mediated regulation of redox balance, mitochondrial dynamics, and nutrient flux may reshape the TME toward immune reactivation (Fig. 1). From a mechanistic perspective, DNAzyme nanocatalysts link molecular recognition to immunometabolic regulation through a stepwise process: (1) sequence-specific RNA cleavage alters the expression of metabolic

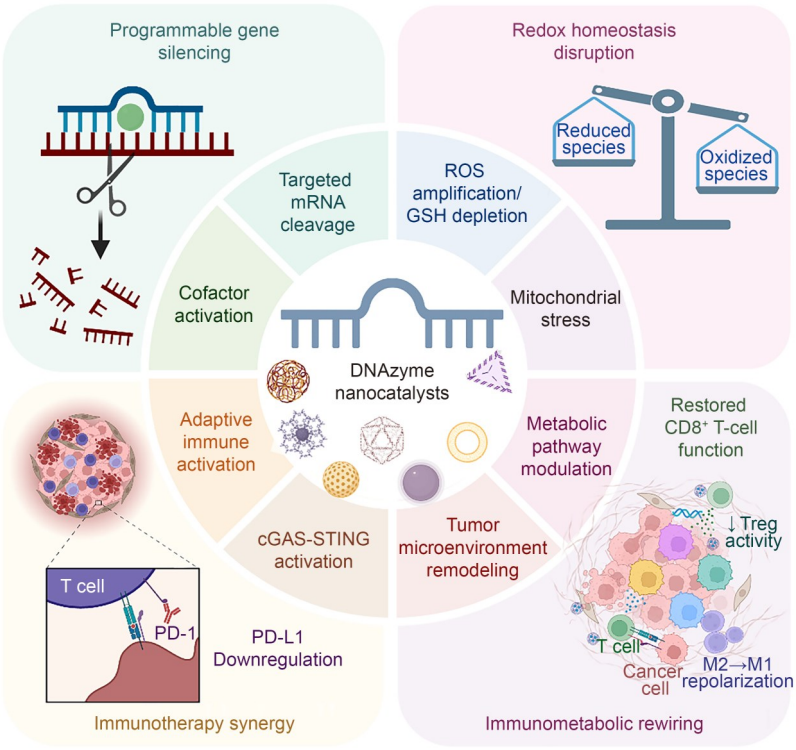

Fig. 1 Mechanistic framework of programmable DNAzyme nanocatalysts for tumor immunometabolic modulation. The DNAzyme-mediated cleavage of selected RNA targets reshapes tumor metabolic pathways, including glycolysis, redox balance, mitochondrial function, and nutrient competition, which in turn restores immune-cell activity and promotes antitumor responses within the tumor microenvironment. ROS: reactive oxygen species; GSH: glutathione; mRNA: messenger RNA; cGAS-STING: cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase-stimulator of interferon genes; PD-1: programmed death protein-1; PD-L1: programmed death-ligand 1; CD8: cluster of differentiation 8; Treg: regulatory T cell. Created with BioRender.com.

or immune-regulatory genes; (2) these transcript-level changes reconfigure redox balance, nutrient competition, mitochondrial activity, and hypoxia adaptation; and (3) the resulting metabolic reprogramming subsequently restores immune-cell function and promotes antitumor immunity in the TME. Overall, programmable RCD nanocatalysts offer a promising framework for connecting catalytic nucleic acid engineering with immunometabolic modulation in cancer-relevant settings.

2 Catalytic mechanisms and engineering principles of RCDs

2.1 Fundamental reaction mechanism and catalytic architecture

RCDs catalyze site-specific RNA phosphodiester cleavage through an intramolecular transesterification reaction that yields a 2',3'-cyclic phosphate and a 5'- hydroxyl terminus (Su et al., 2023). The reaction requires a precise in-line alignment of the 2'-OH nucleophile, the scissile phosphate, and the 5'-oxygen leaving group. Mechanistically, catalysis can be understood through four common contributions: α-catalysis, which promotes the proper in-line geometry; β-catalysis, which stabilizes the negative charge on the non-bridging phosphate oxygens; γ-catalysis, which facilitates deprotonation and activation of the 2'-OH nucleophile; and δ-catalysis, which stabilizes or protonates the 5'-oxygen leaving group. In RCDs, these effects are often assisted by divalent metal ions such as Mg^{2+}, Zn^{2+}, Ca^{2+}, and Mn^{2+}, which contribute to folding, charge compensation, and transition-state stabilization. Structural and spectroscopic studies further indicate that RCD catalytic cores, despite comprising only about 13–15 nucleotides, can fold into compact tertiary architectures that support efficient catalysis (Liu et al., 2017; Wieruszewska et al., 2024; Muñoz-González et al., 2025).

2.2 Representative motifs: 8-17 and 10-23

Among known RCD families, the 8-17 and 10-23 motifs are the best characterized regarding structure, metal dependence, and catalytic reactivity (Santoro and Joyce, 1998; Liu et al., 2017; Rosenbach et al., 2020). The 8-17 DNAzyme contains a compact catalytic core with a conserved AGC triloop and typically cleaves N–G junctions. Structural studies suggest that it adopts a relatively well pre-organized catalytic architecture in which residues such as N14 play a central mechanistic role in promoting cleavage (Fig. 2a) (Liu et al., 2017; Wieruszewska et al., 2024). The 10-23 DNAzyme, by contrast, contains a catalytic loop flanked by two hybridization arms and can cleave nearly any purine–pyrimidine junction. Time-resolved nuclear magnetic resonance (NMR) and simulation studies show that metal-ion binding promotes conformational rearrangement into a reactive state, enabling broader substrate recognition but also a greater dependence on dynamic structural transitions (Fig. 2b) (Borggräfe et al., 2022; Cramer et al., 2023). Thus, the 8-17 DNAzyme functions as a rigid, pre-organized catalyst, whereas the 10-23 DNAzyme relies on conformational plasticity and metal-assisted activation. These motif-specific ion requirements are also highly relevant to nanoplatform design, because scaffold composition and stimuli-responsive cofactor release determine whether catalysis can be sustained in biological environments.

2.3 Sequence engineering, chemical optimization, and mechanistic insights

The conversion of RCDs into biologically competent catalysts requires the rational optimization of both sequence and chemistry, guided by mechanistic understanding. Tuning the length and overall hybridization strength of the substrate-binding arms can influence target accessibility, substrate affinity, catalytic turnover, and product release, whereas chemical modifications can improve nuclease resistance, structural integrity, and biological persistence. Commonly used modifications include 2'-*O*-methyl, phosphorothioate, locked nucleic acid (LNA), and xeno-nucleic acid (XNA) linkages. However, these are not universally

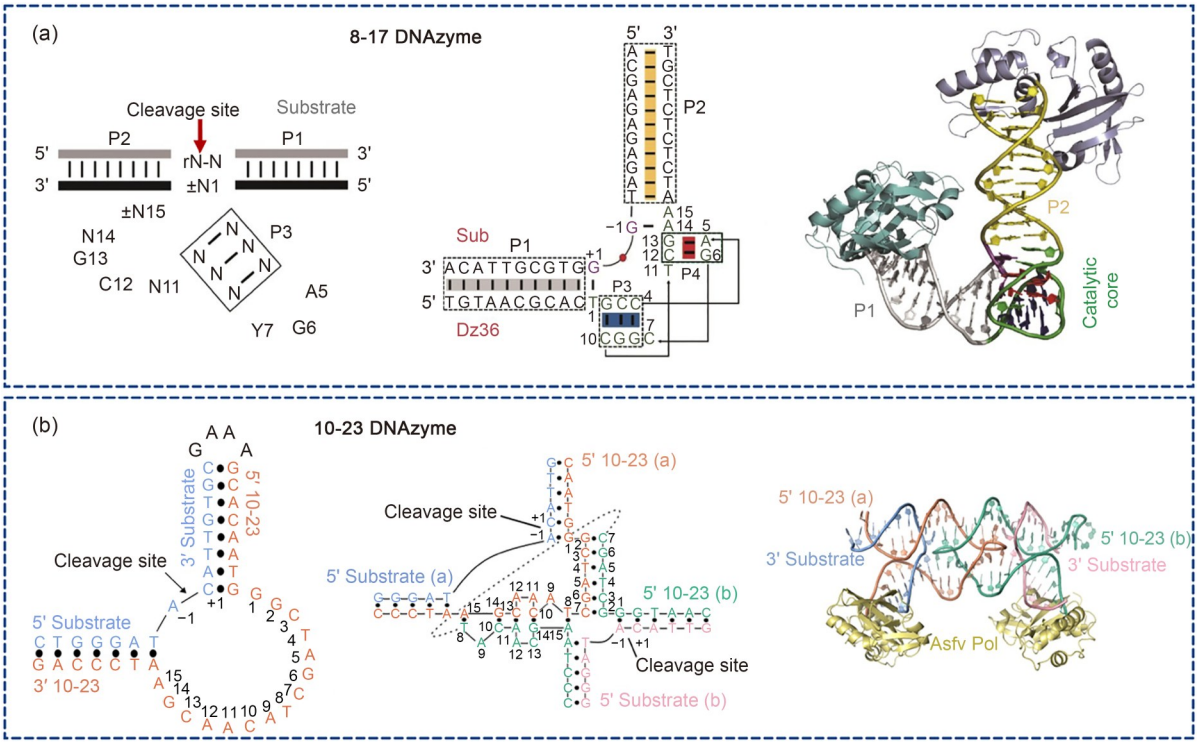

Fig. 2 Representative structural characteristics of the 8-17 and 10-23 DNAzymes. (a) The 8-17 DNAzyme constitutes a representative consensus sequence, secondary structure, and crystal structure of the Pb^{2+}-bound DNAzyme-substrate complex, with AsfvPolX chaperones. Reproduced from Liu et al. (2017). (b) The 10-23 DNAzyme exhibits a secondary structure of the crystallization construct, base-pair interactions supporting catalysis, and corresponding crystal structure with the AsfvPolX chaperone. Reproduced from Cramer et al. (2023).

beneficial and must be deployed in a position-dependent manner. When placed in terminal or recognition regions, they often enhance stability with limited catalytic penalty; in contrast, their incorporation too close to the catalytic core may perturb local folding, metal-ion coordination, substrate positioning, product release, or cleavage efficiency (Wang YJ et al., 2021; Du et al., 2025).

Among the currently available chemistries, 2'-*O*-methyl is often considered relatively well balanced because it improves nuclease resistance and can reduce unwanted biological reactivity while remaining broadly compatible with established oligonucleotide synthesis workflows. Likewise, the phosphorothioate modification enhances stability and biological persistence, while it may also increase nonspecific protein interactions and, when excessively incorporated, compromise catalytic behavior. LNA can markedly increase target affinity and conformational preorganization, yet its high rigidity may reduce turnover or impede product release if introduced near catalytically sensitive regions. XNA-like chemistries may offer even greater biostability, whereas their compatibility with metal-ion-dependent DNAzyme catalysis is better regarded as chemotype-specific than universal.

These trade-offs parallel broader principles established in U.S. Food and Drug Administration (FDA)-approved RNA therapeutics, where 2'-modified sugars and phosphorothioate linkages are widely used because they provide a practical balance among stability, tolerability, and manufacturability. For DNAzymes, however, stabilization alone is insufficient, as catalytic function must also be preserved. Accordingly, the goal of chemical optimization is, rather than maximal modification, the selective placement of chemically advantageous motifs in ways that enhance biostability and reduce immunogenic or pharmacological liabilities while minimizing disruptions to catalytic geometry and function (Nguyen et al., 2023). These mechanistic and chemical design principles provide a basis for integrating RCDs into the nanoplatform architectures discussed in Section 3.

2.4 Cofactor availability in vivo: catalytic thresholds and safety trade-offs

A central limitation of therapeutic RCDs is the mismatch between metal-ion concentrations commonly used for in vitro activation and the much lower free metal-ion availability in physiological settings. As a result, the key translational issue is whether nanocarriers can generate sufficiently high and localized cofactor concentrations in vivo to sustain catalysis without causing off-target toxicity. Existing platforms attempt to solve this problem through stimuli-responsive ion release from ZnO, manganese dioxide (MnO_2), MOFs, or metal-nucleic-acid frameworks, thereby creating transient local enrichment after tumor accumulation or intracellular uptake. However, direct quantitative evidence that such ion release consistently reaches catalytically effective thresholds at the reaction site remains limited. Moreover, strategies that enhance local cofactor supply may also increase the risk of nonspecific redox injury or metal-associated toxicity. Therefore, many current systems are best viewed as partially validated local-activation models rather than fully resolved solutions, highlighting the need for real-time ion mapping, cleavage-cofactor correlation, and more rigorous safety assessment in vivo.

3 Nanoengineering strategies for DNAzyme-based platforms

3.1 Rationale for nanoscale integration

While RCDs can exhibit excellent catalytic efficiency and sequence specificity under controlled in vitro conditions, their performance in biological settings is often constrained by intrinsic physicochemical barriers. Naked DNAzymes are susceptible to nuclease degradation, nonspecific protein adsorption, and poor cellular uptake due to their negatively charged phosphate backbone, while the absence of a stabilizing microenvironment may further impair proper folding and limit local metal-ion availability, thereby reducing in vivo catalytic efficiency (Khan et al., 2021; Liu YF et al., 2022; Chen Y et al., 2025).

Nanoscale integration provides a rational solution to address the above limitations by creating a confined catalytic microenvironment that more closely approximates the spatial organization and cofactor proximity required for efficient catalysis. When DNAzymes are incorporated into nanoscale carriers or hybrid architectures, the surrounding matrix can simultaneously protect them from serum instability, support structural integrity, enrich catalytic metal ions such as Zn^{2+}, Mn^{2+}, or Mg^{2+}, and enable conditionally biased activation through aptamers, peptides, or cleavable linkers responsive to tumor-associated cues such as acidic pH, redox imbalance, or microRNA overexpression (Wang J

et al., 2019; Khan et al., 2021; Liu et al., 2021). Through nanoconfinement, cofactor self-supply, and stimuli-responsive design, DNAzymes are thus transformed from free catalytic oligonucleotides into programmable nanocatalysts with improved potential for spatially and temporally controlled activity in complex biological environments. These principles form the basis for the development of diverse DNAzyme nanoplatforms, including metal-oxide, DNA-framework, and MOF-based systems, each of which offers distinct advantages and translational trade-offs for catalytic control and therapeutic modulation.

3.2 Engineered nanoplatform architectures for DNAzyme integration

The selection of an appropriate carrier determines the assembly strategy, activation mechanism, and overall biological behavior of DNAzyme systems. The subsections below summarize representative nanoplatforms that sustain catalytic activity under physiological stress.

3.2.1 Metal-oxide scaffolds: intrinsic cofactor reservoirs

Metal-oxide nanostructures provide robust and catalytically active frameworks that stabilize DNAzymes while regulating the redox environment of tumors. Their large surface area and metal-rich lattice make them self-replenishing cofactor sources, ensuring sustained activation under physiological stress (Zhang et al., 2025).

ZnO is a prototypical scaffold in which acidic dissolution releases Zn^{2+} ions, simultaneously restoring catalytic activity and amplifying redox signaling. ZnO generally remains comparatively stable under near-neutral physiological conditions (around pH 7.4), whereas dissolution becomes more evident under mildly acidic conditions and markedly accelerates below approximately pH 6.5, particularly in the pH 5.0–6.0 range of endosomal or lysosomal compartments. Given that the extracellular TME is typically mildly acidic (commonly with pH 6.5–6.9), ZnO-based systems can undergo partial destabilization after tumor accumulation, while more efficient Zn^{2+} liberation often occurs after cellular internalization into acidic vesicles (Mu et al., 2025; Yuan et al., 2025). This pH-responsive behavior provides a physiologically relevant basis for tumor-selective cofactor release and DNAzyme activation.

Chen et al. (2024) developed self-catabolic DNAzyme nanospheres (aptamer (Apt)-self-catabolic DNAzyme nanosphere (SCNS)-doxorubicin (Dox)-ZnO) that couple acid-triggered Zn^{2+} release with DNAzyme activation, achieving synergistic gene silencing and oxidative stress enhancement for tumor suppression. The liberated Zn^{2+} not only activates the embedded DNAzyme but also contributes to redox disequilibrium and reactive oxygen species (ROS) amplification within tumor cells, establishing a self-reinforcing gene/redox therapeutic loop. Building on this concept, manganese-based scaffolds introduce an additional immunometabolic dimension. MnO_2 frameworks release Mn^{2+} ions under reductive conditions, triggering both DNAzyme activation and oxygen generation via hydrogen peroxide (H_2O_2) decomposition, which alleviates hypoxia and promotes immunogenic cell death (ICD) (Du SY et al., 2022). These systems exemplify how metal-oxide nanoscaffolds can integrate catalytic, metabolic, and immune functions, transforming inert carriers into multifunctional nanoreactors capable of orchestrating gene silencing, oxidative modulation, and immune reprogramming within the TME.

3.2.2 DNA-framework nanostructures: spatial precision and molecular logic

DNA nanotechnology enables the bottom-up construction of well-defined nanostructures with nanometer-level spatial precision and predictable stoichiometry. Unlike amorphous carriers, DNA-framework nanostructures, including TDNs, rolling circle amplification (RCA) hydrogels, and hierarchical nanogels, provide rigid, programmable architectures that preserve DNAzyme folding, enhance molecular recognition, and support controllable activation in response to tumor-associated cues. The inherent biocompatibility and structural modularity of these nanostructures make them particularly suitable for intracellular delivery and logic-gated DNAzyme catalysis.

TDNs illustrate how geometric precision directly enhances catalytic fidelity: their rigid tetrahedral scaffold protects DNAzymes from nuclease degradation, minimizes conformational fluctuations, and presents catalytic motifs multivalently at defined vertices to improve substrate accessibility. Surface modification with aptamers or peptide ligands enables receptor-mediated uptake, while pH-, redox-, or microRNA-responsive linkers allow controlled intracellular activation (Meng et al., 2019; Zhang et al., 2021). These structures can be validated at multiple levels; for example, molecular assays (e.g., fluorescence and Forster resonance energy

transfer [FRET]) are used to confirm logic-gated activation, polyacrylamide gel electrophoresis (PAGE) assays can validate strand displacement and structural rearrangements, and cellular imaging confirms that activation occurs only in the presence of the intended cellular triggers (e.g., specific microRNAs or redox conditions).

Yu et al. (2022) designed a toehold-triggered TDN nanomachine in which the catalytic core remained sequestered until the recognition of intracellular miR-21. Target binding induced a structural rearrangement, exposing the active site for selective messenger RNA (mRNA) cleavage (Fig. 3a). Zhao S et al. (2025) extended this concept with a trinity DNAzyme circuit (TriDC), using a TDN loaded with MnO_2 nanosheets to create an AND-gate response activated by miR-10b and miR-155. This circuit also promotes chemodynamic therapy by releasing Mn^{2+} ions from MnO_2 nanosheets, generating ROS to kill tumor cells (Fig. 3b). These DNA-framework platforms integrate spatial precision with biocompatibility and stimuli-responsiveness, enabling efficient intracellular delivery and tumor-targeted gene regulation.

3.2.3 Metal-organic frameworks and redox-responsive hybrids: crystalline catalytic nanoreactors

MOFs offer crystalline, porous architectures that are ideal for protecting and activating DNAzymes. Their tunable pore networks and metal coordination sites support high loading capacity and continuous cofactor supply, enabling controlled, environment-responsive catalysis in vivo (Zhu et al., 2023). Wang Z et al. (2021) designed a bimetallic Cu/Zn zeolitic imidazolate framework-8 (ZIF-8) framework in which Zn^{2+} served as a DNAzyme cofactor while Cu^{+} generated cytotoxic species through Fenton-like reactions, allowing coordinated gene silencing and chemodynamic therapy. The same group later extended this strategy to construct a

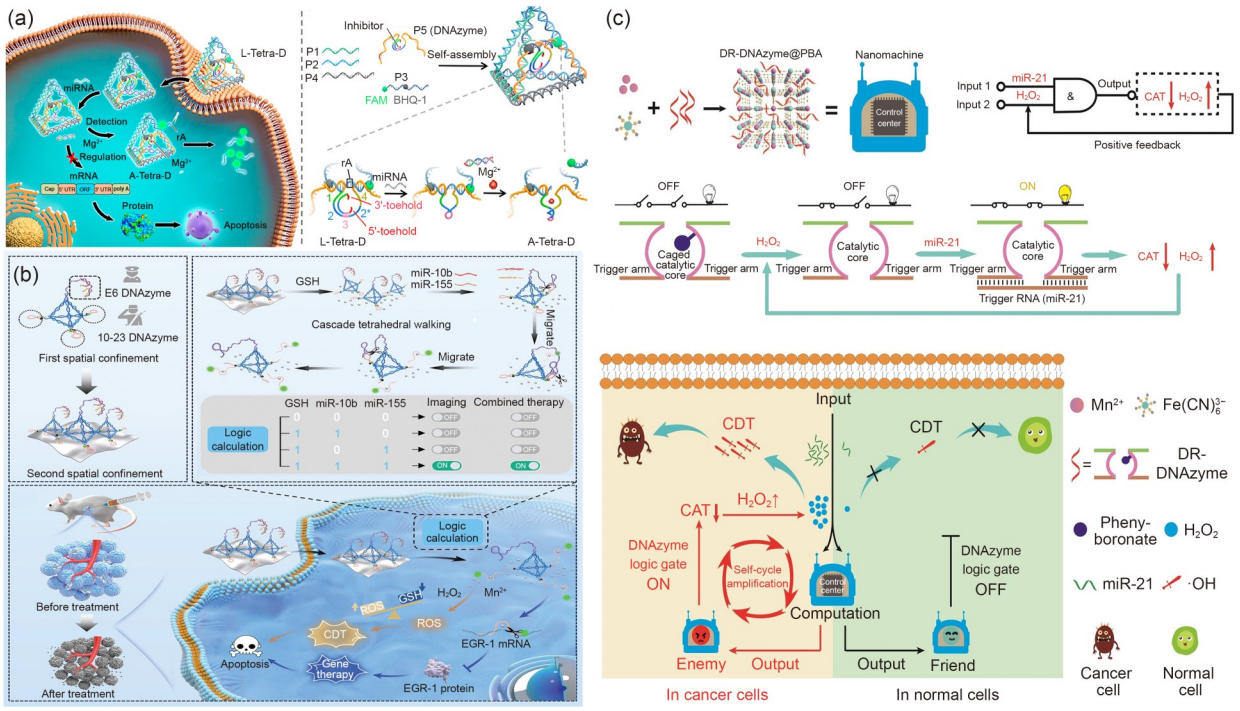

Fig. 3 Engineered nanoplatforms for DNAzyme integration. (a) DNA tetrahedral nanomachine enabling miRNA-responsive activation through toehold-mediated strand displacement for precise intracellular sensing and regulation. UTR: untranslated region; ORF: open reading frame; FAM: 6-carboxyfluorescein; BHQ1: black hole quencher-1. Reproduced from Yu et al. (2022), Copyright 2022, with permission from Elsevier. (b) Trinity DNAzyme circuit (TriDC) platform combining DNAzyme catalysis and cofactor generation for imaging-guided, tumor-selective chemodynamic therapy. GSH: glutathione; ROS: reactive oxygen species; CDT: chemical dynamic therapy; EGR-1: early growth response protein 1. Reproduced from Zhao S et al. (2025), Copyright 2025, with permission from John Wiley and Sons. (c) DNAzyme logic nanomachine operating via an AND-gate mechanism, where miR-21 and H_2O_2 inputs uncage the catalytic core to trigger cell-specific CDT while remaining inactive in normal cells. DR-DNAzyme: dual-responsive DNAzyme; PBA: Prussian blue analogue; CAT: catalase. Reproduced from Wang et al. (2022), Copyright 2022, with permission from John Wiley and Sons.

logic-gated DNAzyme circuit embedded in a Prussian blue analogue MOF, where intracellular redox cues triggered catalase-mRNA cleavage and enhanced ROS amplification, ensuring tumor-selective activation (Wang et al., 2022) (Fig. 3c). Similarly, Li et al. (2020) reported a ZIF-82 core-shell system where pH-responsive Zn^{2+} release and glutathione (GSH) depletion induced redox collapse, enhancing DNAzyme-mediated catalysis and oxidative stress.

3.2.4 Critical comparison of platform classes and translational bottlenecks

Although metal-oxide, DNA-framework, and MOF-based scaffolds have all advanced DNAzyme delivery and activation, their translational strengths and limitations differ markedly. Metal-oxide platforms offer relatively simple fabrication, intrinsic cofactor supply, and efficient stimuli-responsive disassembly, but may suffer from premature dissolution, less precise structural uniformity, and potential ion-associated toxicity, all of which potentially affect batch reproducibility. DNA-framework nanostructures provide superior programmability and structural precision, making them attractive for logic-gated activation and mechanistic control; however, they remain vulnerable to nuclease degradation, structural perturbation in physiological fluids, and relatively high synthesis and purification costs. MOF-based systems combine high loading capacity, tunable porosity, and multifunctional integration, yet their translational development is often constrained by framework heterogeneity, variable stability in complex biological media, and difficulties in achieving consistent large-scale production. To summarize the key differences and limitations of the three core nanoplatform architectures discussed in this section, Table 1 provides a direct comparison of their composition, catalytic cofactors, activation mechanisms, advantages, and limitations. This allows for a clearer understanding of the distinct features and potential applications of each platform, helping to identify the most suitable platform for specific therapeutic needs. Overall, no single platform is uniformly optimal across all translational metrics: while metal-oxide systems favor simplicity and self-supplied activation, DNA frameworks excel in programmability, and MOFs are advantageous for cargo integration; however, reproducibility, physiological robustness, and scalable manufacturing remain common bottlenecks that require further standardization.

3.3 Delivery barriers and limits of stimulus selectivity

Although stimuli-responsive engineering has improved the apparent controllability of DNAzyme nanoplatforms, the central bottleneck of these systems remains delivery rather than activation alone (Fan et al., 2023). In vivo efficacy is constrained by sequential barriers operating at the tissue, cellular, and subcellular levels, including limited tumor penetration caused by a dense extracellular matrix and elevated interstitial fluid pressure, heterogeneous accumulation despite the partial contribution of the enhanced permeability and retention (EPR) effect, inefficient endosomal escape, and progressive endo-lysosomal sequestration (Rennick et al., 2021; Zhao Y et al., 2023). Moreover, tumor-associated biochemical cues are rarely binary or tumor-exclusive but instead exist as overlapping spatial gradients that may also occur in inflamed normal tissues, thereby limiting the practical selectivity of current logic-gated designs (Jing et al., 2022). Therefore, future DNAzyme nanoplatforms should move beyond stimulus responsiveness alone and instead emphasize integrated

Table 1 Comparison of three key DNAzyme nanoplatforms

Nanoplatform	Activation mechanism	Advantages	Limitations	References
Metal-oxide scaffold	pH-responsive, redox-activated	Simple synthesis, intrinsic cofactor support, efficient catalytic activation	Premature dissolution, lack of structural uniformity, toxicity risks	Du SY et al., 2022; Chen et al., 2024; Zhang et al., 2025
DNA-framework	Logic-gated, substrate-responsive	High programmability, structural precision, sequence-specific activation	Nuclease degradation, complex synthesis, limited in vivo stability	Meng et al., 2019; Zhang et al., 2021; Yu et al., 2022; Zhao S et al., 2025
Metal-organic framework (MOF)	pH-triggered, redox-activated, co-delivery of multiple agents	High cargo loading capacity, multifunctional, potential for co-delivery of drugs/imaging agents	Instability, batch-to-batch variability, scale-up production challenges	Wang HM et al., 2019; Li et al., 2020; Wang Z et al., 2021, 2022; Zhu et al., 2023

solutions that combine penetrability, cytosolic accessibility, biodegradability, and stricter multi-factor recognition for more reliable in vivo catalysis.

4 DNAzyme-driven immunometabolic therapy

4.1 Concept and rationale

Recent nanotechnology advances have widened the role of DNAzymes from sequence-specific gene silencers to dynamic regulators of tumor immunometabolism. Within the metabolically disordered TME, cancer cells rely heavily on aerobic glycolysis and glutaminolysis, generating excess lactate, acidosis, and ROS. These changes in turn impair mitochondrial respiration in effector immune cells, promote adenosine triphosphate (ATP) exhaustion, and support immunosuppressive cell populations such as TAMs and Tregs (Binnewies et al., 2018; Guo YD et al., 2024; Su et al., 2024). The resulting biochemical imbalance establishes an immune-resistant niche that limits responsiveness to immunotherapy. To counteract these barriers, DNAzyme-based nanocatalysts offer a programmable strategy. Their catalytic activity toward specific RNA targets can be integrated with metal-ion-mediated redox modulation, enabling simultaneous gene regulation and metabolic remodeling (Zhao X et al., 2022; Zhao ZH et al., 2025). Additionally, DNAzyme-mediated reprogramming of metabolic pathways can influence the activity of immunosuppressive populations like M2-like TAMs, myeloid-derived suppressor cells (MDSCs), and Tregs (Dong et al., 2023; Guo JL et al., 2024). By modulating nutrient availability, including glucose and glutamine, these systems may weaken the metabolic support that sustains immunosuppressive functions in these cells, thereby reactivating immune responses.

4.2 Redox reprogramming and mitochondrial restoration

DNAzyme-based nanocatalysts provide a platform to modulate this redox-mitochondrial axis in a coordinated manner. When metal ions released from nanocarriers activate DNAzyme catalysis, they also participate in redox reactions that weaken tumor antioxidant defenses. Wu et al. (2022) developed a Zn^{2+}-responsive DNAzyme embedded in ZIF-8. In acidic TME conditions, Zn^{2+} release was shown to inhibit glycolytic enzymes, deplete nicotinamide adenine dinucleotide (NAD^+), and activate DNAzyme cleavage of glucose transporter 1 (*GLUT1*) mRNA, thereby blocking glucose uptake. The combined effects induced ATP depletion, ROS accumulation, and mitochondrial depolarization, thereby collapsing the metabolic reserve of the tumor (Fig. 4a). Furthermore, Yan et al. (2024) designed a Ca^{2+}-dependent DNA framework that combined DNAzyme-mediated *GLUT1* silencing with reduced nicotinamide adenine dinucleotide phosphate (NADPH) and GSH synthesis, while Ca^{2+} overload triggered mitochondrial permeability transition. Elevated ROS, membrane-potential loss, and mitochondrial swelling shifted tumor cells from an energy-rich, reductive phenotype to a metabolically exhausted and immunogenic state (Fig. 4b).

In addition to altering tumor metabolism, DNAzyme nanocatalysts may help reverse T-cell exhaustion by re-energizing these immune cells. By restoring oxidative phosphorylation and reducing metabolic stress in T cells, these platforms could alleviate chronic T-cell exhaustion and restore their cytotoxic potential (Zhu et al., 2023). Thus, the combined modulation of redox homeostasis and immune signaling enables DNAzyme nanoplatforms to provide a comprehensive therapeutic effect on both tumor cells and immune cells.

4.3 Targeted gene silencing for immune modulation

Besides metabolic modulation, DNAzyme nanoplatforms can regulate immune signaling pathways to counteract immune evasion. Guo et al. (2023) developed a core-shell nanomedicine (DPCPX@Dz) combining an adenosine A1-receptor inhibitor (8-cyclopentyl-1,3-dipropylxanthine [DPCPX]) with an anti-programmed death-ligand 1 (anti-PD-L1) DNAzyme (Dz). While adenosine blockade initiated ICD, it also triggered compensatory PD-L1 upregulation. The DNAzyme alleviated this feedback by cleaving *PD-L1* mRNA, restoring T-cell cytotoxicity, and producing durable tumor regression (Fig. 5a). Chen et al. (2023) engineered a MnO_2-adjuvant DNAzyme system (H/LDz-M@B, a nanosystem in which the hairpin DNA and locked-DNAzyme [LDz] were electrostatically adsorbed on MnO_2 and then encapsulated with bovine serum albumin [BSA]) that activated cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase-stimulator of interferon genes (cGAS-STING) via a miR-21-initiated hybridization chain reaction and simultaneously silenced *PD-L1* through

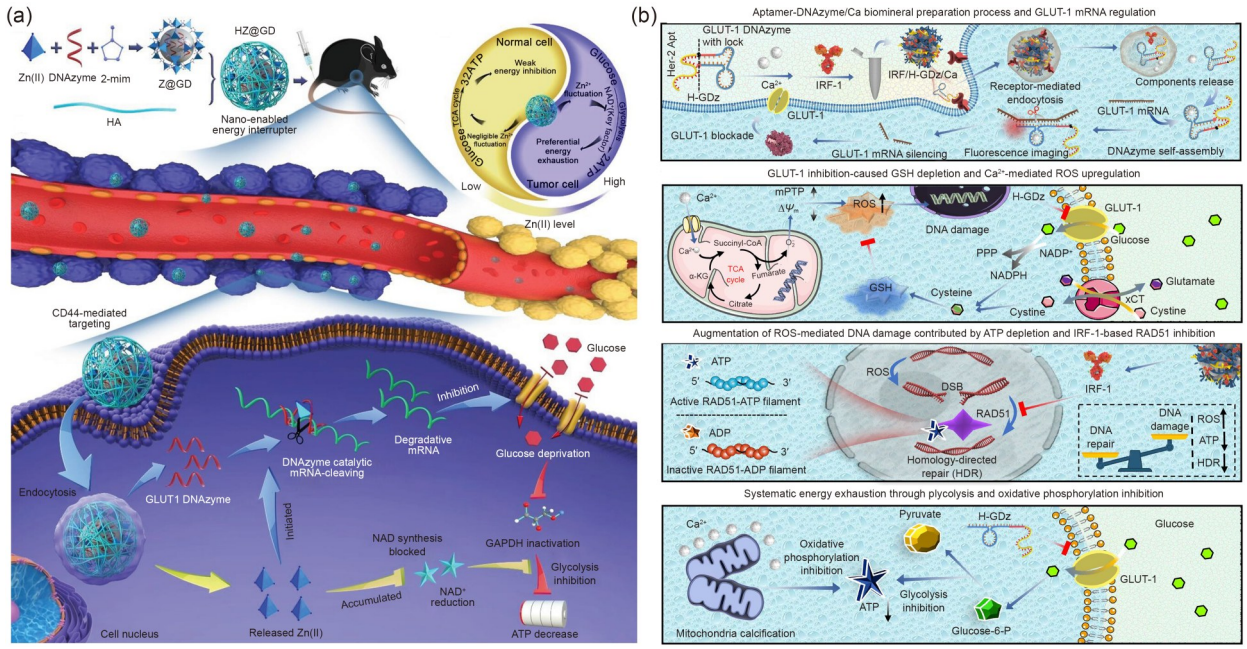

Fig. 4 DNAzyme nanoplatforms for redox modulation and mitochondrial restoration. (a) Schematic of the Zn^{2+}/DNAzyme "energy-interrupter" nanoplatform. GLUT1: glucose transporter 1; Z: ZIF-8; HZ: hyaluronic acid (HA)-modified ZIF-8; GD: a highly efficient Zn^{2+}-activating GLUT1 mRNA-cleaving DNAzyme; ATP: adenosine triphosphate; TCA: tricarboxylic acid cycle; CD44: cluster of differentiation 44; NAD: nicotinamide adenine dinucleotide; GAPDH: glyceraldehyde-3-phosphate dehydrogenase. Reproduced from Wu et al. (2022). (b) Metal-nucleic-acid frameworks for redox and mitochondrial regulation. GLUT1 suppression disturbs the glutathione (GSH)/reactive oxygen species (ROS) balance, promotes mitochondrial Ca^{2+} overload and membrane-permeability transition, and reduces ATP production. H-GRz: the sequence comprising 41 nucleotides represented GLUT1 DNAzymes with a fluorescence lock, referred to as GDz, and the sequence consisting of 83 nucleotides corresponded to GDz extended by HER-2 aptamer, denoted as H-GDz; IRF-1: interferon regulatory factor-1; mPTP: mitochondrial permeability transition pore; ψ_m: mitochondrial membrane potential; $NADP^+$: nicotinamide adenine dinucleotide phosphate; NADPH: reduced nicotinamide adenine dinucleotide phosphate; xCT: cystine/glutamate antiporter SLC7A11; PPP: pentose phosphate pathway; CoA: coenzyme A; α-KG: alpha-ketoglutarate; ADP: adenosine diphosphate; DSB: double-strand break. Reproduced from Yan et al. (2024).

miR-146a-responsive DNAzymes. This dual pathway enhanced interferon-β (IFN-β) production, dendritic cell maturation, and T-cell recruitment (Fig. 5b). Xu et al. (2024) introduced a ZnO-Au@$mSiO_2$ (ZnO-mesoporous silica-encapsulated gold) radio-immunoenhancer in which X-ray-triggered Zn^{2+} release activated *PD-L1*-cleaving DNAzymes and the cGAS-STING axis. This synergy strengthened ICD, boosted IFN-β and tumor necrosis factor-α (TNF-α) secretion, and enhanced T-cell infiltration in non-small cell lung cancer (NSCLC) (Fig. 5c). Chen C et al. (2025) further expanded this concept by developing a near-infrared (NIR)-triggered upconversion nanoparticle (UCNP)-MnO_2 nanomachine for the co-delivery of clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9 ribonucleoproteins (Cas9 RNPs) and DNAzymes. Light-induced MnO_2 decomposition activated *PD-L1* silencing and CRISPR editing concurrently, producing robust innate-immune activation and redox normalization in hypoxic tumors (Fig. 5d).

Collectively, these studies highlight a unifying mechanism: the DNAzyme-mediated mRNA cleavage of immune-regulatory genes, combined with metal-assisted redox cues, activates innate immunity and overcomes checkpoint-mediated resistance. Such programmable systems enable precise immune modulation and enhance the effectiveness of immunotherapy.

4.4 Mechanistic convergence and therapeutic outlook

Across diverse nanoplatforms, DNAzyme nanocatalysts demonstrate a convergent mechanism in which localized catalytic activation initiates coordinated redox, metabolic, and immune transformations within the TME. Compared with immune checkpoint inhibitors, RNA therapeutics such as small interfering RNA

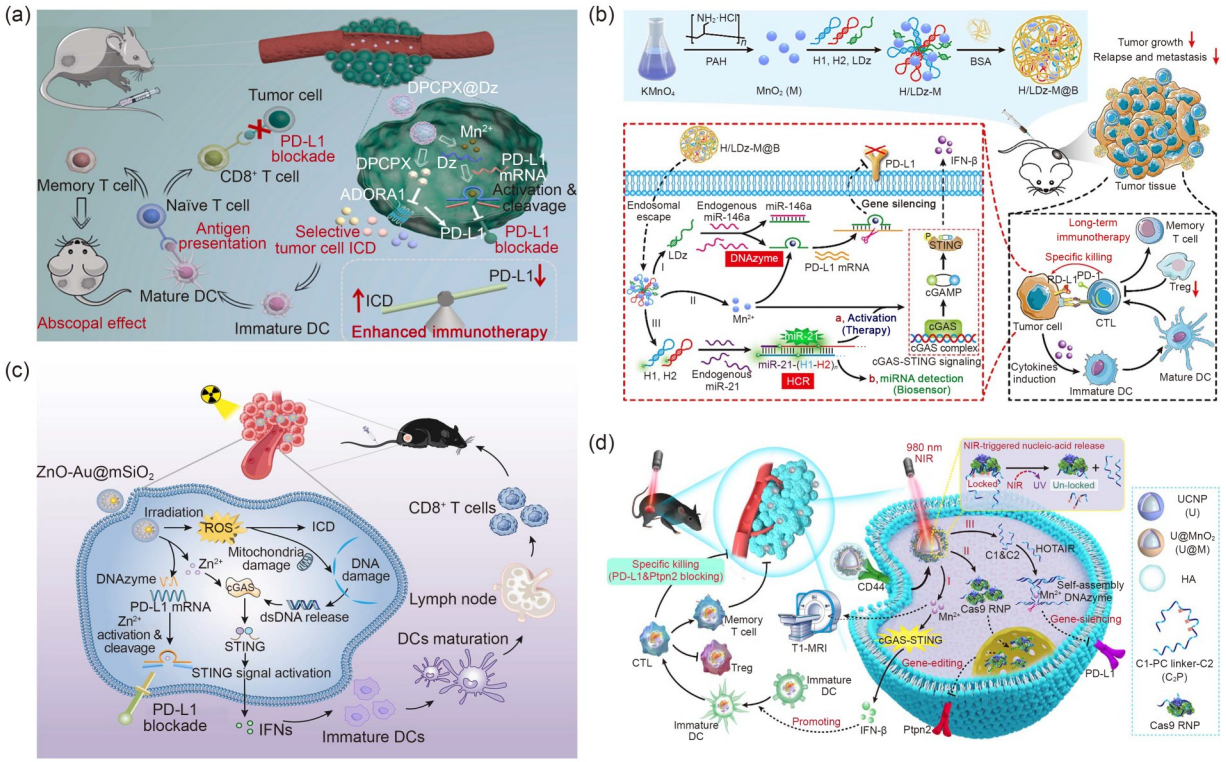

Fig. 5 DNAzyme nanoplatforms for targeted immune modulation. (a) DPCPX@Dz nanoplatform that induces immunogenic cell death (ICD) through adenosine A1 receptor (ADORA1) inhibition while Mn^{2+}-activated programmed death-ligand 1 (PD-L1) DNAzyme (Dz) downregulates PD-L1 to enhance T cell-mediated immunity. DPCPX: 8-cyclopentyl-1,3-dipropylxanthine; DC: dendritic cell. Reproduced from Guo et al. (2023), Copyright 2023, with permission from Elsevier. (b) MicroRNA (miRNA)-triggered theranostic system that activates cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase-stimulator of interferon genes (cGAS-STING) signaling and *PD-L1* messenger RNA (mRNA) cleavage via dual DNAzyme circuits. PAH: polycyclic aromatic hydrocarbons; LDz: locked-DNAzyme; BSA: bovine serum albumin; H1 and H2: hairpin DNAs; cGAMP: cyclic GMP-AMP; HCR: hybridization chain reaction; Treg: regulatory T cell; PD-1: programmed death protein-1; CTL: cytotoxic T lymphocyte; IFN: interferon. Reproduced from Chen et al. (2023), Copyright 2023, with permission from Elsevier. (c) ZnO-Au@mSiO$_2$ nanoparticles enabling X-ray-enhanced ICD and Zn^{2+}-activated DNAzyme/cGAS-STING pathways for amplified immune response. Au@mSiO$_2$: mesoporous silica-encapsulated gold; ROS: reactive oxygen species; dsDNA: double-stranded DNA. Reproduced from Xu et al. (2024). (d) NIR-responsive UCNP-MnO$_2$ nanomachine co-delivering Cas9 ribonucleoproteins (RNPs) and DNAzymes for light-controlled *PD-L1* silencing and immune activation. HA: hyaluronic acid; C1: Chain 1; C2: Chain 2; PC: photocleavable; UCNP: upconversion nanoparticle; U@M: manganese-modified UCNPs; NIR: near-infrared; UV: ultraviolet; Cas9: clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9; Ptpn2: protein tyrosine phosphatase non-receptor (PTPN) type 2; MRI: magnetic resonance imaging; HOTAIR: HOX transcript antisense intergenic RNA. Reproduced from Chen C et al. (2025).

(siRNA), antisense oligonucleotides (ASOs), CRISPR-Cas systems, and DNAzyme nanocatalysts exhibit distinct mechanistic advantages. While checkpoint inhibitors primarily act through the systemic blockade of immune checkpoint pathways, DNAzyme-based platforms can couple immune checkpoint regulation with direct metabolic remodeling, enabling broader immunometabolic reprogramming (Zhang and Zhu, 2026). In contrast to siRNA and ASOs, which depend on endogenous RNA interference (RNAi) or RNase H machinery, DNAzymes perform sequence-specific RNA cleavage via an intrinsic catalytic mechanism, offering a potential edge in integrating with cofactor-responsive and logic-gated platforms (Wang J et al., 2019). In addition, compared to CRISPR-Cas systems, DNAzymes provide a reversible, transient modulation of gene expression, bypassing the permanent genome-editing concerns inherent to CRISPR approaches (Yan et al., 2023). However, these potential advantages remain largely preclinical, and hence DNAzyme systems must still overcome challenges related to delivery, stability, and clinical validation.

Future progress will rely on developing system-level programmable designs with multi-input logic control, optimized cofactor stoichiometry, and improved biodegradability. Integrating DNAzyme catalysis with checkpoint blockade, phototherapy, or metabolic inhibitors may further enhance therapeutic responses. Representative systems are summarized in Table 2, which compares platform composition, catalytic cofactors, molecular targets, immunometabolic effects, reported therapeutic outcomes, and key translational challenges, thereby providing a more practical overview of both the promise and current limitations of DNAzyme-based nanomedicine. By uniting molecular precision with adaptive biological feedback, programmable DNAzyme nanoplatforms hold considerable promise as future therapeutics for immunometabolic intervention.

5 Toxicological, pharmacokinetic, and off-target risks in DNAzyme nanoplatform translation

Beyond catalytic design, the clinical translation of DNAzyme nanoplatforms is strongly limited by toxicological and pharmacokinetic barriers. Particularly for inorganic or poorly biodegradable scaffolds, incomplete clearance and predominant uptake by the mononuclear phagocyte system can lead to accumulation in the liver and spleen, raising concerns about long-term retention and chronic toxicity (Fan et al., 2023). DNA-based nanostructures can also engage innate immune sensing pathways, including endosomal Toll-like receptor 9 (TLR9), in a manner that depends on cytidine-phosphate-guanosine (CpG) dinucleotide presentation, sequence context, chemical stabilization, and intracellular trafficking (Du RR et al., 2022). In parallel, many nanoplatforms exhibit suboptimal pharmacokinetics, including protein corona formation, rapid reticuloendothelial system (RES) clearance, and low tumor delivery efficiency, which collectively constrain effective intracellular delivery (Wang J et al., 2021). These challenges underscore the need for rigorous long-term biodistribution studies, standardized immunotoxicity assessment, and delivery optimization that can emphasize biodegradability, stealth behavior, and improved tumor access.

Moreover, although RCDs exhibit high sequence specificity under controlled in vitro conditions, their functional specificity in vivo is influenced by the complexity of the intracellular and tissue environments. Variations in cofactor availability, competitive nucleic acid interactions, RNA structural accessibility, and non-specific uptake by off-target cells can all compromise effective target discrimination, increasing the risk of unintended activity. While many studies report successful target gene silencing and therapeutic benefits, transcriptome-wide in vivo analyses of DNAzyme off-target effects remain limited. This highlights the need for more systematic safety assessments, ideally involving transcriptome-level profiling, mismatch discrimination analysis, and context-dependent selectivity testing. To mitigate these risks, molecular engineering strategies, including selective chemical modifications, multi-input logic-gated activation, localized cofactor release, and tumor-specific delivery, are actively being explored. These approaches aim to suppress basal activity, improve target selectivity, and confine DNAzyme activation to the intended cellular context, which will be critical for enhancing both the safety and therapeutic efficacy of DNAzyme-based nanoplatforms.

6 Conclusions and perspectives

The integration of DNAzyme catalysis with nanoscale engineering has established a new class of stimuli-responsive nanoplatforms for sensing and modulating tumor immunometabolism. By combining the sequence specificity of RCDs with the structural programmability of engineered nanomaterials, these systems extend beyond conventional redox modulators or gene-silencing agents: they can function as biosensing nanocatalytic systems that interpret local biochemical cues and convert them into coordinated catalytic responses, thereby influencing metabolic pathways, redox balance, and immune signaling within the TME.

Despite promising developments, several challenges must be addressed before these platforms can move closer to translation. Improving in vivo stability, pharmacokinetics and biosafety, particularly for metal-based constructs, remains crucial for predictable catalytic performance and reducing platform-associated toxicity. In addition to these nanocarrier-related risks, the sequence-dependent off-target RNA cleavage of DNAzymes should be systematically assessed in vivo through transcriptome-level profiling and mismatch-discrimination analyses. Hierarchical and logic-gated nanostructures that selectively respond to combinations of endogenous stimuli may further improve conditional

Table 2 Representative programmable DNAzyme nanoplatforms for tumor immunometabolic modulation, featuring therapeutic outcomes and translational challenges

Nanoplatform	Cofactor	Target	Immunometabolic effects	Therapeutic outcomes	Translational challenges	Reference
MnO_2-based Ce6-modified DNAzyme	Mn^{2+}	*PD-L1* mRNA	cGAS-STING activation; checkpoint relief; enhanced antitumor immunity	Tumor suppression; immune activation	Mn-related safety; limited long-term biosafety and pharmacokinetic evaluation	Du SY et al., 2022
Mn/Fe metal-phenolic network	Mn^{2+}	*PD-L1* mRNA	ICD induction; IFN-γ release; CTL reactivation	Ferroptosis-immunotherapy synergy	Formulation complexity; off-target toxicity risk	Liu P et al., 2022
ALA/DNAzyme@ZIF-8	Zn^{2+}	*FECH* mRNA	Hypoxia relief; mtDNA release; cGAS-STING/IFN-β activation	Enhanced PDT-immunotherapy	Light dependence; limited deep-tumor applicability	Zhao X et al., 2023
Cell-membrane-coated Zn/MnO_2 nanoflowers	Mn^{2+}	*ASCT2* mRNA	Dual glutamine/glucose restriction; redox disruption; cGAS-STING activation	Strong tumor inhibition; T-cell infiltration	Complex structure; limited advanced-model validation	Guo JL et al., 2024
ZnO-Au@$mSiO_2$	Zn^{2+}	*PD-L1* mRNA	Oxidative stress enhancement; ICD; cGAS-STING activation	Improved radio immunotherapy	X-ray dependence; composite safety concerns	Xu et al., 2024
Mn virus-mimicking nanomedicine	Mn^{2+}	miR-19a/PTEN	cGAS-STING activation; STAT3 inhibition; Treg reduction	Anti-metastatic efficacy	Brain-delivery translation uncertainty	Zhao ZH et al., 2025
Herpesvirus-mimicking Mn-doped ZIF-90	Zn^{2+}	*TFAM* mRNA	mtDNA stress; innate immune activation; cGAS-STING signaling	Tumor suppression; immune activation	Regulatory complexity; biodistribution unclear	Zhao et al., 2022
Aptamer-DNAzyme metal framework	Ca^{2+}	*GLUT1* mRNA	GSH/ROS imbalance; mitochondrial stress; enhanced DNA-damage response	Metabolic exhaustion; tumor inhibition	Limited in vivo validation; degradability unclear	Yan et al., 2024
HA-ZIF-8/DNAzyme	Zn^{2+}	*GLUT1* mRNA	Glycolysis inhibition; NAD^+ depletion; tumor energy exhaustion	Energy exhaustion; tumor inhibition	Zn off-target risk; long-term safety unknown	Wu et al., 2022
DPCPX@Dz core-shell nanomedicine	Mn^{2+}	*PD-L1* mRNA	ADORA1 blockade-induced ICD; checkpoint relief; DC and $CD8^+$ T-cell activation	Durable regression; immune memory	Mechanistic complexity; co-delivery standardization needed	Guo et al., 2023
MnO_2/HCR DNAzyme systems	Mn^{2+}	miR-21/*PD-L1* mRNA	Cytoplasmic dsDNA generation; cGAS-STING activation; adaptive immune amplification	Long-term immune response	Circuit complexity; manufacturability	Chen et al., 2023
NIR upconversion DNAzyme/Cas9 nanomedicine	Mn^{2+}	*PD-L1* mRNA/Cas9	cGAS-STING activation; dual checkpoint modulation; TME remodeling	Strong systemic antitumor immunity	Editing safety; high translational complexity	Chen C et al., 2025

Ce6: chlorin e6; PD-L1: programmed death-ligand 1; cGAS-STING: cyclic guanosine monophosphate-adenosine monophosphate (GMP-AMP) synthase-stimulator of interferon genes; ICD: immunogenic cell death; IFN: interferon; CTL: cytotoxic T lymphocyte; ALA: 5-aminolevulinic acid; ZIF-8: zeolitic imidazolate framework-8; ALA/DNAzyme@ZIF-8: ALA and DNAzyme-loaded ZIF-8; FECH: ferrochelatase; mtDNA: mitochondrial DNA; PDT: photodynamic therapy; ASCT2: alanine/serine/cysteine transporter 2; Au@$mSiO_2$: mesoporous silica-encapsulated gold; PTEN: phosphatase and tensin homolog; STAT3: signal transducer and activator of transcription 3; Treg: regulatory T cell; TFAM: transcription factor A, mitochondrial; GLUT1: glucose transporter 1; GSH: glutathione; ROS: reactive oxygen species; HA: hyaluronic acid; NAD: nicotinamide adenine dinucleotide; DPCPX: 8-cyclopentyl-1,3-dipropylxanthine; Dz: DNAzyme; ADORA1: adenosine A1 receptor; DC: dendritic cell; HCR: hybridization chain reaction; dsDNA: double-stranded DNA; NIR: near-infrared; Cas9: clustered regularly interspaced short palindromic repeats (CRISPR)-associated protein 9; TME: tumor microenvironment.

activation, restrict DNAzyme activity to the intended cellular context, and reduce systemic exposure. In parallel, future studies should quantitatively determine whether stimuli-responsive ion release can generate and sustain catalytically effective local cofactor concentrations in vivo without inducing metal-associated off-target toxicity.

At the same time, the clinical relevance of current DNAzyme nanoplatforms should not be overstated, as most available evidence remains limited to murine models. Translation to human disease is complicated by the greater heterogeneity of clinical tumors, unresolved Chemistry, Manufacturing, and Controls (CMC) challenges for complex multifunctional formulations, and stringent regulatory requirements for inorganic nanoparticle-based therapeutics, particularly regarding scale-up reproducibility, long-term biodistribution, biodegradation, and organ-retention safety. Thus, future progress will require more clinically relevant tumor models, stronger manufacturing standardization, and more rigorous long-term safety evaluation.

Looking forward, integrating DNAzyme nanocatalysts with complementary therapeutic modalities, metabolic inhibition, and nucleic-acid logic circuits offers exciting opportunities for synergistic and durable tumor control. Such integrated strategies may amplify catalytic events, enhance immune priming, and overcome metabolic barriers that currently limit immunotherapeutic efficacy.

In summary, programmable DNAzyme nanocatalysts provide a valuable framework for embedding biosensing principles into therapeutic nanomaterials, linking molecular recognition with metabolic and immune modulation within a single platform. Continued interdisciplinary innovation in chemical biology, materials science, immunometabolism, and bioengineering will help advance the development of more robust and potentially translatable DNAzyme systems for future cancer theranostics and related biomedical applications.

Acknowledgments

This work was supported by the Seeking Truthful Talents Projects of Hangzhou Medical College (No. 00004E1RCYJ2408), the Natural Science Foundation of Hangzhou (No. 2025SZRJJ0037), the General Research Project of the Zhejiang Provincial Department of Education (No. Y202559865), and the Joint Funds of the National Natural Science Foundation of China (No. U22A20342 to J.L.).

Author contributions

Rongping LUO and Zhuojia TANG: literature collection, writing – original draft, and visualization. Mengqi DING and Lingxiu ZOU: literature collection and writing – review & editing. Xiaojing LIU: literature collection and manuscript revision. Fan YIN: writing – review & editing. Jianxin LYU: supervision, project administration, and funding acquisition. Lu WANG: conceptualization, writing – review & editing, supervision, project administration, and funding acquisition. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Compliance with ethics guidelines

Rongping LUO, Zhuojia TANG, Mengqi DING, Lingxiu ZOU, Xiaojing LIU, Fan YIN, Jianxin LYU, and Lu WANG declare that they have no conflicts of interest.

This review does not contain any studies with human or animal subjects performed by any of the authors.

Declaration on the use of generative AI tools

During the preparation of this work, the authors used Grammarly to improve language and readability. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

Binnewies M, Roberts EW, Kersten K, et al., 2018. Understanding the tumor immune microenvironment (TIME) for effective therapy. *Nat Med*, 24(5):541-550. https://doi.org/10.1038/s41591-018-0014-x

Borggräfe J, Victor J, Rosenbach H, et al., 2022. Time-resolved structural analysis of an RNA-cleaving DNA catalyst. *Nature*, 601(7891):144-149. https://doi.org/10.1038/s41586-021-04225-4

Cai WQ, Liu J, Chen XH, et al., 2022. Orthogonal chemical activation of enzyme-inducible CRISPR/Cas9 for cell-selective genome editing. *J Am Chem Soc*, 144(48):22272-22280. https://doi.org/10.1021/jacs.2c10545

Chen C, Du SY, Lu QL, et al., 2023. Tumor-associated miRNAs activated HCR-DNAzyme theranostic nanosystem to trigger innate- and adaptive-immune responses for long-term immunotherapy. *Chem Eng J*, 473:145192. https://doi.org/10.1016/j.cej.2023.145192

Chen C, Du SY, Lu QL, et al., 2025. Spatiotemporally delivery of Cas9 ribonucleoprotein/DNAzyme logic systems using near-infrared upconversion nanomachine for precise immunotherapy. *Acta Pharm Sin B*, 15(10):5431-5443. https://doi.org/10.1016/j.apsb.2025.07.010

Chen Y, Guo Y, Wang JL, et al., 2024. Engineering self-catabolic DNAzyme nanospheres for synergistic anticancer therapy. *Sci China Chem*, 67(7):2412-2422. https://doi.org/10.1007/s11426-024-2095-6

Chen Y, Guo ZK, Li J, et al., 2025. Smart nucleic acid nanodrug delivery system for precision therapeutics. *Coord Chem Rev*, 535:216673.

https://doi.org/10.1016/j.ccr.2025.216673

Cramer ER, Starcovic SA, Avey RM, et al., 2023. Structure of a 10-23 deoxyribozyme exhibiting a homodimer conformation. *Commun Chem*, 6:119. https://doi.org/10.1038/s42004-023-00924-3

Dong X, Xia S, Du SB, et al., 2023. Tumor metabolism-rewriting nanomedicines for cancer immunotherapy. *ACS Cent Sci*, 9(10):1864-1893. https://doi.org/10.1021/acscentsci.3c00702

Du RR, Cedrone E, Romanov A, et al., 2022. Innate immune stimulation using 3D wireframe DNA origami. *ACS Nano*, 16(12):20340-20352. https://doi.org/10.1021/acsnano.2c06275

Du SY, Chen C, Qu SC, et al., 2022. DNAzyme-assisted nanoherb delivery system for multiple tumor immune activation. *Small*, 18(45):2203942. https://doi.org/10.1002/smll.202203942

Du Z, Wu XQ, Dang Y, et al., 2025. Molecular bioengineering of functional nucleic acids. *Nat Rev Bioeng*, 4(2):153-170. https://doi.org/10.1038/s44222-025-00361-y

Fan DH, Cao YK, Cao MQ, et al., 2023. Nanomedicine in cancer therapy. *Sig Transduct Target Ther*, 8:293. https://doi.org/10.1038/s41392-023-01536-y

Guo J, Liu P, Wei BL, et al., 2023. Reversing the negative effect of adenosine A1 receptor-targeted immunometabolism modulation on melanoma by a co-delivery nanomedicine for self-activation of anti-PD-L1 DNAzyme. *Nano Today*, 48:101722. https://doi.org/10.1016/j.nantod.2022.101722

Guo JL, Wu LG, Liu CX, et al., 2024. DNAzyme-loaded bimetallic nanoflowers for STING activation and dual metabolic regulation to enhance antitumor immunity. *Chem Eng J*, 501:157697. https://doi.org/10.1016/j.cej.2024.157697

Guo YD, Hu P, Shi JL, 2024. Nanomedicine remodels tumor microenvironment for solid tumor immunotherapy. *J Am Chem Soc*, 146(15):10217-10233. https://doi.org/10.1021/jacs.3c14005

Huang K, Han Y, Chen YH, et al., 2025. Tumor metabolic regulators: key drivers of metabolic reprogramming and the promising targets in cancer therapy. *Mol Cancer*, 24:7. https://doi.org/10.1186/s12943-024-02205-6

Jing XD, Hu H, Sun YZ, et al., 2022. The intracellular and extracellular microenvironment of tumor site: the trigger of stimuli-responsive drug delivery systems. *Small Methods*, 6(3):2101437. https://doi.org/10.1002/smtd.202101437

Jouha J, Xiong H, 2021. DNAzyme-functionalized nanomaterials: recent preparation, current applications, and future challenges. *Small*, 17(51):2105439. https://doi.org/10.1002/smll.202105439

Kao KC, Vilbois S, Tsai CH, et al., 2022. Metabolic communication in the tumour–immune microenvironment. *Nat Cell Biol*, 24(11):1574-1583. https://doi.org/10.1038/s41556-022-01002-x

Kaymak I, Williams KS, Cantor JR, et al., 2021. Immunometabolic interplay in the tumor microenvironment. *Cancer Cell*, 39(1):28-37. https://doi.org/10.1016/j.ccell.2020.09.004

Khan S, Burciu B, Filipe CDM, et al., 2021. DNAzyme-based biosensors: immobilization strategies, applications, and future prospective. *ACS Nano*, 15(9):13943-13969. https://doi.org/10.1021/acsnano.1c04327

Li F, Yu WT, Zhang JJ, et al., 2021. Spatiotemporally programmable cascade hybridization of hairpin DNA in polymeric nanoframework for precise siRNA delivery. *Nat Commun*, 12:1138. https://doi.org/10.1038/s41467-021-21442-7

Li F, Song NC, Dong YH, et al., 2022. A proton-activatable DNA-based nanosystem enables co-delivery of CRISPR/Cas9 and DNAzyme for combined gene therapy. *Angew Chem Int Ed*, 61(9):e202116569. https://doi.org/10.1002/anie.202116569

Li XY, Gao JJ, Wu CC, et al., 2024. Precise modulation and use of reactive oxygen species for immunotherapy. *Sci Adv*, 10(20):eadl0479. https://doi.org/10.1126/sciadv.adl0479

Li YL, Zhao PR, Gong T, et al., 2020. Redox dyshomeostasis strategy for hypoxic tumor therapy based on DNAzyme-loaded electrophilic ZIFs. *Angew Chem Int Ed*, 59(50):22537-22543. https://doi.org/10.1002/anie.202003653

Liu CZ, Chen YX, Zhao J, et al., 2021. Self-assembly of copper-DNAzyme nanohybrids for dual-catalytic tumor therapy. *Angew Chem Int Ed*, 60(26):14324-14328. https://doi.org/10.1002/anie.202101744

Liu HH, Yu X, Chen YQ, et al., 2017. Crystal structure of an RNA-cleaving DNAzyme. *Nat Commun*, 8:2006. https://doi.org/10.1038/s41467-017-02203-x

Liu P, Shi XY, Peng Y, et al., 2022. Anti-PD-L1 DNAzyme loaded photothermal Mn^{2+}/Fe^{3+} hybrid metal-phenolic networks for cyclically amplified tumor ferroptosis-immunotherapy. *Adv Healthcare Mater*, 11(8):2102315. https://doi.org/10.1002/ADHM.202102315

Liu SW, Liu ZJ, Lei HJ, et al., 2025. Programmable nanomodulators for precision therapy, engineering tumor metabolism to enhance therapeutic efficacy. *Adv Healthcare Mater*, 14(4):2403019. https://doi.org/10.1002/adhm.202403019

Liu YF, Zhu P, Huang J, et al., 2022. Integrating DNA nanostructures with DNAzymes for biosensing, bioimaging and cancer therapy. *Coord Chem Rev*, 468:214651. https://doi.org/10.1016/j.ccr.2022.214651

Meng LX, Ma WJ, Lin SY, et al., 2019. Tetrahedral DNA nanostructure-delivered DNAzyme for gene silencing to suppress cell growth. *ACS Appl Mater Interfaces*, 11(7):6850-6857. https://doi.org/10.1021/acsami.8b22444

Mu S, Yang HY, Wang SQ, et al., 2025. Zinc-based nanomaterials in cancer therapy: mechanisms, applications, and future directions. *Theranostics*, 15(15):7841-7871. https://doi.org/10.7150/thno.117773

Muñoz-González M, Silva-Galleguillos V, Parra-Meneses V, et al., 2025. Catalytic mechanisms of the 8-17 and 10-23 DNAzymes: shared mechanistic strategies. *Org Biomol Chem*, 23(19):4564-4577. https://doi.org/10.1039/D5OB00387C

Nguyen K, Malik TN, Chaput JC, 2023. Chemical evolution of an autonomous DNAzyme with allele-specific gene silencing activity. *Nat Commun*, 14:2413. https://doi.org/10.1038/s41467-023-38100-9

Norian LA, Rodriguez PC, O'Mara LA, et al., 2009. Tumor-infiltrating regulatory dendritic cells inhibit $CD8^+$ T cell function via L-arginine metabolism. *Cancer Res*, 69(7): 3086-3094. https://doi.org/10.1158/0008-5472.CAN-08-2826

Rennick JJ, Johnston APR, Parton RG, 2021. Key principles and methods for studying the endocytosis of biological and nanoparticle therapeutics. *Nat Nanotechnol*, 16(3):266-276. https://doi.org/10.1038/s41565-021-00858-8

Rosenbach H, Victor J, Etzkorn M, et al., 2020. Molecular features and metal ions that influence 10-23 DNAzyme activity. *Molecules*, 25(13):3100. https://doi.org/10.3390/molecules25133100

Santoro SW, Joyce GF, 1998. Mechanism and utility of an RNA-cleaving DNA enzyme. *Biochemistry*, 37(38):13330-13342. https://doi.org/10.1021/bi9812221

Scharping NE, Rivadeneira DB, Menk AV, et al., 2021. Mitochondrial stress induced by continuous stimulation under hypoxia rapidly drives T cell exhaustion. *Nat Immunol*, 22(2):205-215. https://doi.org/10.1038/s41590-020-00834-9

Shi XX, Meng HM, Geng X, et al., 2020. DNAzyme–metal–organic framework two-photon nanoprobe for in situ monitoring of apoptosis-associated Zn^{2+} in living cells and tissues. *ACS Sens*, 5(10):3150-3157. https://doi.org/10.1021/acssensors.0c01271

Su JX, Sun CY, Du JY, et al., 2023. RNA-cleaving DNAzyme-based amplification strategies for biosensing and therapy. *Adv Healthcare Mater*, 12(23):2300367. https://doi.org/10.1002/adhm.202300367

Su RR, Shao YY, Huang MR, et al., 2024. Immunometabolism in cancer: basic mechanisms and new targeting strategy. *Cell Death Discov*, 10:236. https://doi.org/10.1038/s41420-024-02006-2

Tan YY, Li JJ, Zhao GY, et al., 2022. Metabolic reprogramming from glycolysis to fatty acid uptake and beta-oxidation in platinum-resistant cancer cells. *Nat Commun*, 13:4554. https://doi.org/10.1038/s41467-022-32101-w

Wang F, Liu ZC, Liu YC, et al., 2024. A spatiotemporally controlled gene-regulation strategy for combined tumor therapy based on upconversion hybrid nanosystem. *Adv Sci*, 11(40):2405640. https://doi.org/10.1002/advs.202405640

Wang HM, Chen YQ, Wang H, et al., 2019. DNAzyme-loaded metal–organic frameworks (MOFs) for self-sufficient gene therapy. *Angew Chem Int Ed*, 58(22):7380-7384. https://doi.org/10.1002/anie.201902714

Wang J, Wang HM, Wang H, et al., 2019. Nonviolent self-catabolic DNAzyme nanosponges for smart anticancer drug delivery. *ACS Nano*, 13(5):5852-5863. https://doi.org/10.1021/acsnano.9b01589

Wang J, Li YY, Nie GJ, 2021. Multifunctional biomolecule nanostructures for cancer therapy. *Nat Rev Mater*, 6(9): 766-783. https://doi.org/10.1038/s41578-021-00315-x

Wang L, Tang SQ, Li L, et al., 2023. Nucleic acids enabled-interfacial engineering for biomarker sensing with distance constraint effects. *Adv Sensor Res*, 2(4):2200062. https://doi.org/10.1002/adsr.202200062

Wang R, He WH, Yi X, et al., 2023. Site-specific bioorthogonal activation of DNAzymes for on-demand gene therapy. *J Am Chem Soc*, 145(32):17926-17935. https://doi.org/10.1021/jacs.3c05413

Wang YJ, Nguyen K, Spitale RC, et al., 2021. A biologically stable DNAzyme that efficiently silences gene expression in cells. *Nat Chem*, 13(4):319-326. https://doi.org/10.1038/s41557-021-00645-x

Wang Z, Niu JS, Zhao CQ, et al., 2021. A bimetallic metal–organic framework encapsulated with DNAzyme for intracellular drug synthesis and self-sufficient gene therapy. *Angew Chem Int Ed*, 60(22):12431-12437. https://doi.org/10.1002/anie.202016442

Wang Z, Yang J, Qin G, et al., 2022. An intelligent nanomachine guided by DNAzyme logic system for precise chemodynamic therapy. *Angew Chem Int Ed*, 61(38):e202204291. https://doi.org/10.1002/anie.202204291

Wieruszewska J, Pawłowicz A, Połomska E, et al., 2024. The 8-17 DNAzyme can operate in a single active structure regardless of metal ion cofactor. *Nat Commun*, 15:4218. https://doi.org/10.1038/s41467-024-48638-x

Wu SX, Zhang KX, Liang Y, et al., 2022. Nano-enabled tumor systematic energy exhaustion via zinc(II) interference mediated glycolysis inhibition and specific GLUT1 depletion. *Adv Sci*, 9(7):2103534. https://doi.org/10.1002/advs.202103534

Xu MJ, Xu C, Qiu Y, et al., 2024. Zinc-based radioenhancers to activate tumor radioimmunotherapy by PD-L1 and cGAS-STING pathway. *J Nanobiotechnol*, 22:782. https://doi.org/10.1186/s12951-024-02999-z

Yan JQ, Ran MX, Shen X, et al., 2023. Therapeutic DNAzymes: from structure design to clinical applications. *Adv Mater*, 35(30):2300374. https://doi.org/10.1002/adma.202300374

Yan JQ, Bhadane R, Ran MX, et al., 2024. Development of Aptamer-DNAzyme based metal-nucleic acid frameworks for gastric cancer therapy. *Nat Commun*, 15:3684. https://doi.org/10.1038/s41467-024-48149-9

Yu LY, Yang S, Liu ZY, et al., 2022. Programming a DNA tetrahedral nanomachine as an integrative tool for intracellular microRNA biosensing and stimulus-unlocked target regulation. *Mater Today Bio*, 15:100276. https://doi.org/10.1016/j.mtbio.2022.100276

Yuan HM, Jiao ZC, Wang T, et al., 2025. Endogenously activated

multiple DNAzyme-encoded nanoflowers for high-contrast imaging of long noncoding RNA and cancer therapy. *ACS Nano*, 19(41):36397-36410. https://doi.org/10.1021/acsnano.5c10251

Zhang HB, Zhu QB, 2026. Recent advances in DNAzyme systems for disease diagnosis and therapy. *Adv Healthcare Mater*, 15(6):e04385. https://doi.org/10.1002/adhm.202504385

Zhang LX, Diao QJ, Li B, et al., 2025. Delivery system of nanozymes loaded with DNAzymes and hypoxia-activated prodrug for anti-tumor metastasis. *Small*, 21(36):2504778. https://doi.org/10.1002/smll.202504778

Zhang P, Ouyang Y, Sohn YS, et al., 2021. pH- and miRNA-responsive DNA-tetrahedra/metal–organic framework conjugates: functional sense-and-treat carriers. *ACS Nano*, 15(4):6645-6657. https://doi.org/10.1021/acsnano.0c09996

Zhao S, Wang XS, Deng RJ, et al., 2025. A trinity DNAzyme circuit with dual-confinement effect for imaging-guided combined gene/chemo-dynamic therapy. *Adv Funct Mater*, 35(23):2422309. https://doi.org/10.1002/adfm.202422309

Zhao X, Wang YY, Jiang WX, et al., 2022. Herpesvirus-mimicking DNAzyme-loaded nanoparticles as a mitochondrial DNA stress inducer to activate innate immunity for tumor therapy. *Adv Mater*, 34(37):2204585. https://doi.org/10.1002/adma.202204585

Zhao X, Cheng H, Wang QW, et al., 2023. Regulating photosensitizer metabolism with DNAzyme-loaded nanoparticles for amplified mitochondria-targeting photodynamic immunotherapy. *ACS Nano*, 17(14):13746-13759. https://doi.org/10.1021/acsnano.3c03308

Zhao Y, Ye ZF, Song DH, et al., 2023. Nanomechanical action opens endo-lysosomal compartments. *Nat Commun*, 14: 6645. https://doi.org/10.1038/s41467-023-42280-9

Zhao ZH, Zhou JY, Li XW, et al., 2025. Manganese-based virus-mimicking nanomedicine with triple immunomodulatory functions inhibits breast cancer brain metastasis. *Biomaterials*, 320:123262. https://doi.org/10.1016/j.biomaterials.2025.123262

Zheng X, Mund A, Mann M, 2025. Deciphering functional tumor-immune crosstalk through highly multiplexed imaging and deep visual proteomics. *Mol Cell*, 85(5):1008-1023.e7. https://doi.org/10.1016/j.molcel.2024.12.023

Zhu XG, Xu JQ, Ling GX, et al., 2023. Tunable metal–organic frameworks assist in catalyzing DNAzymes with amplification platforms for biomedical applications. *Chem Soc Rev*, 52(21):7549-7578. https://doi.org/10.1039/D3CS00386H