Review
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Programmable DNAzyme nanocatalysts for tumor immunometabolic modulation

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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 been traditionally explored for targeted gene regulation, recent advances in nanotechnology have repositioned them as programmable biosensing modules with stimulus-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 stimulus-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 DNAzymes; DNAzyme nanocatalysts; programmable biosensing; tumor immunometabolism; stimuli-responsive nanoplatforms

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1 Introduction

Tumor immunometabolism refers to the intricate interplay between cellular metabolic pathways and immune regulation within the tumor microenvironment (TME) (Kao et al., 2022; Kaymak et al., 2021; 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 (Huang et al., 2025; Tan et al., 2022). 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 bioenergetic flexibility, while regulatory T cells and tumor-associated macrophages 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 more 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, or gene-editing tools operate with limited spatial and conditional control and may not respond efficiently to fluctuating biochemical conditions (F. Li et al., 2021; F. 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, stimulus-responsive biosensing principles offer a useful conceptual framework for designing therapeutic systems that couple molecular recognition with localized action (X. Li et al., 2024; S. Liu et al., 2025; Z. Wang et al., 2022).

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 & 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 (H. Liu et al., 2017; L. Wang 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 ZnO nanoparticles (Y. Chen et al., 2024; F. Li et al., 2022; J. Wang et al., 2019), DNA tetrahedra (TNDs) (Yu et al., 2022; S. Zhao et al., 2025), lipid vesicles (Cai et al., 2022; R. Wang et al., 2023), or metal-organic frameworks (MOF) (Shi et al., 2020; H. H. Wang et al., 2019; Z. Wang 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.

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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 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 RNA-cleaving DNAzyme nanocatalysts offer a promising framework for connecting catalytic nucleic-acid engineering with immunometabolic modulation in cancer-relevant settings.

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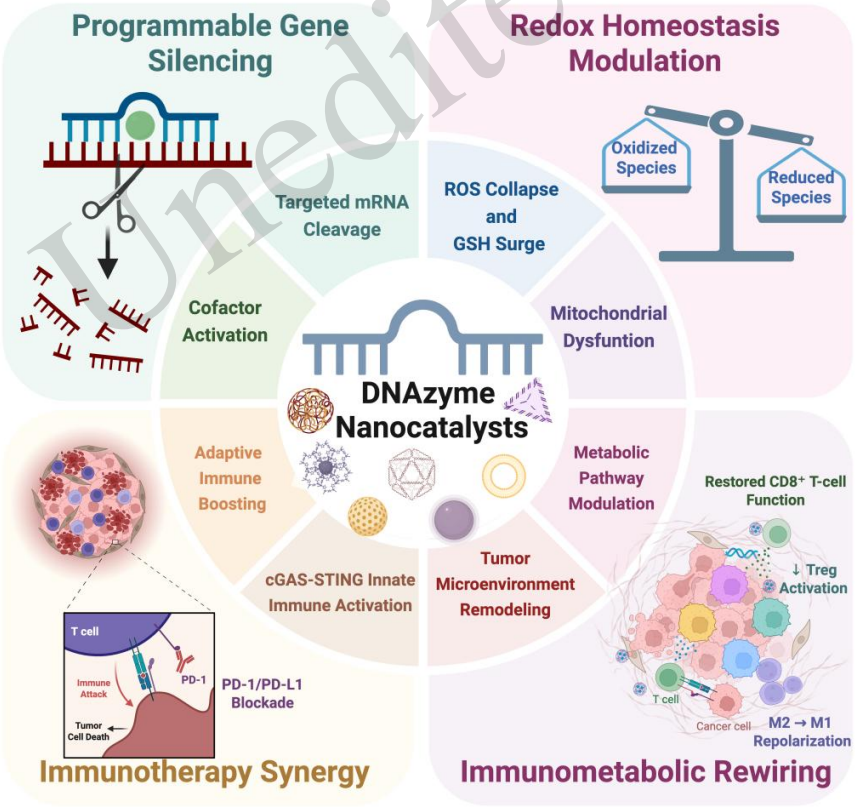

Fig. 1 Mechanistic framework of programmable DNAzyme nanocatalysts for tumor immunometabolic modulation. The DNAzyme-mediated cleavage of selected RNA targets reshapes tumor metabolic pathways, including glycolysis, redox balance, mitochondrial function, and nutrient competition, which in turn restores immune-cell activity and promotes antitumor responses within the tumor microenvironment. Created with BioRender.com.

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 (J. 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 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 RNA-cleaving DNAzymes, 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 (H. Liu et al., 2017; Muñoz-González et al., 2025; Wieruszewska et al., 2024).

2.2 Representative motifs: 8-17 and 10-23

Among known RCDs families, the 8-17 and 10-23 motifs are best characterized regarding structure, metal dependence and catalytic reactivity (H. Liu et al., 2017; Rosenbach et al., 2020; Santoro & Joyce, 1998). 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 G14 play a central mechanistic role in promoting cleavage (H. Liu et al., 2017; Wieruszewska et al., 2024) (Fig. 2a). 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 NMR and simulation studies show that metal-ion binding promotes conformational rearrangement into a reactive state, enabling broader substrate recognition but also greater dependence on dynamic structural transitions (Borggräfe et al., 2022; Cramer et al., 2023) (Fig. 2b). Thus, 8-17 functions as a rigid, pre-organized catalyst, whereas 10-23 relies on conformational plasticity and metal-assisted activation.These motif-specific ion requirements are also highly relevant for nanoplatform design, because scaffold composition and stimulus-responsive cofactor release determine whether catalysis can be sustained in biological environments.

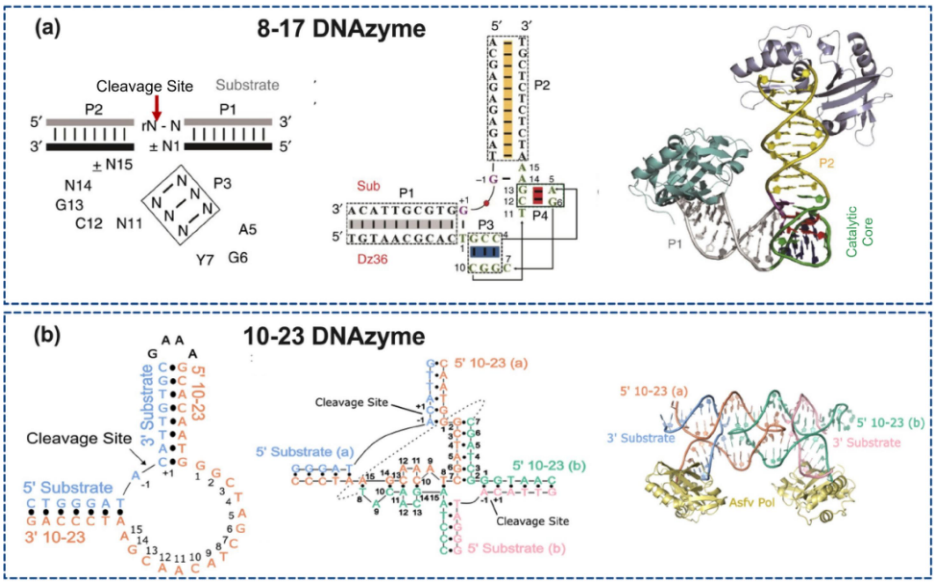

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Fig. 2 Representative structural characteristics of the 8-17 and 10-23 DNAzymes. (a) 8-17 DNAzyme constitutes a representative consensus sequence, secondary structure, and crystal structure of the Pb^{2+}-bound DNAzyme-substrate complex, with AsfvPolX chaperones shown in light blue. Reproduced under the terms of the CC BY license. Copyright 2017, Springer Nature (H. Liu et al., 2017). (b) 10-23 DNAzyme is a secondary structure of the crystallization construct, base-pair interactions supporting catalysis, and corresponding crystal structure with the AsfvPolX chaperone Reproduced under the terms of the CC BY license. Copyright 2023, Springer Nature.(Cramer et al., 2023).

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), or xeno-nucleic-acid (XNA) linkages. However, these are not universally 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, incorporation too close to the catalytic core may perturb local folding, metal-ion coordination, substrate positioning, product release, or cleavage efficiency (Z. Du et al., 2025; Y. Wang et al., 2021).

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 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 of 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 RNA-cleaving DNAzymes 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 stimulus-responsive ion release from ZnO, 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*.

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3. Nanoengineering strategies for DNAzyme-based platforms

3.1 The 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 (Y. Chen et al., 2025; Khan et al., 2021; Y. Liu et al., 2022).

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 (Khan et al., 2021; C. Liu et al., 2021; J. Wang et al., 2019). 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 metal-organic framework (MOF)-based systems, each of which offering distinct advantages and translational trade-offs for catalytic control and therapeutic modulation.

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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, sustaining 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 (L. Zhang et al., 2025).

Zinc oxide (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/lysosomal compartments. Given that the extracellular tumor microenvironment 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. developed self-catabolic DNAzyme nanospheres (Apt-SCNS-Dox-ZnO) that couple acid-triggered Zn^{2+} release with DNAzyme activation, achieving synergistic gene silencing and oxidative stress

enhancement for tumor suppression (Y. Chen et al., 2024). 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 H_2O_2 decomposition, which alleviates hypoxia and promotes immunogenic cell death (S. Du 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-amplified (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; P. Zhang et al., 2021). These structures can be validated at multiple levels, such as molecular assays (e.g., fluorescence, FRET) are used to confirm logic-gated activation, 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 miRNAs or redox conditions).

Yu et al. designed a toehold-triggered TDN nanomachine in which the catalytic core remained sequestered until the recognition of intracellular miR-21 (Yu et al., 2022). Target binding induced a structural rearrangement, exposing the active site for selective mRNA cleavage (Fig 3a). Zhao et al. extended this concept with a tripartite DNAzyme circuit, 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 (S. Zhao et al., 2025) (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

Metal-organic frameworks (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 et al. designed a bimetallic Cu/Zn ZIF-8 framework in which Zn^{2+} served as DNAzyme cofactor while Cu^{+} generated cytotoxic species through Fenton-like reactions, allowing coordinated gene silencing and chemodynamic therapy (Z. Wang et al., 2021). The authors later same group extended this strategy to construct a 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 (Z. Wang et al., 2022) (Fig. 3c). Similarly, Li et al. 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 (Y. Li et al., 2020).

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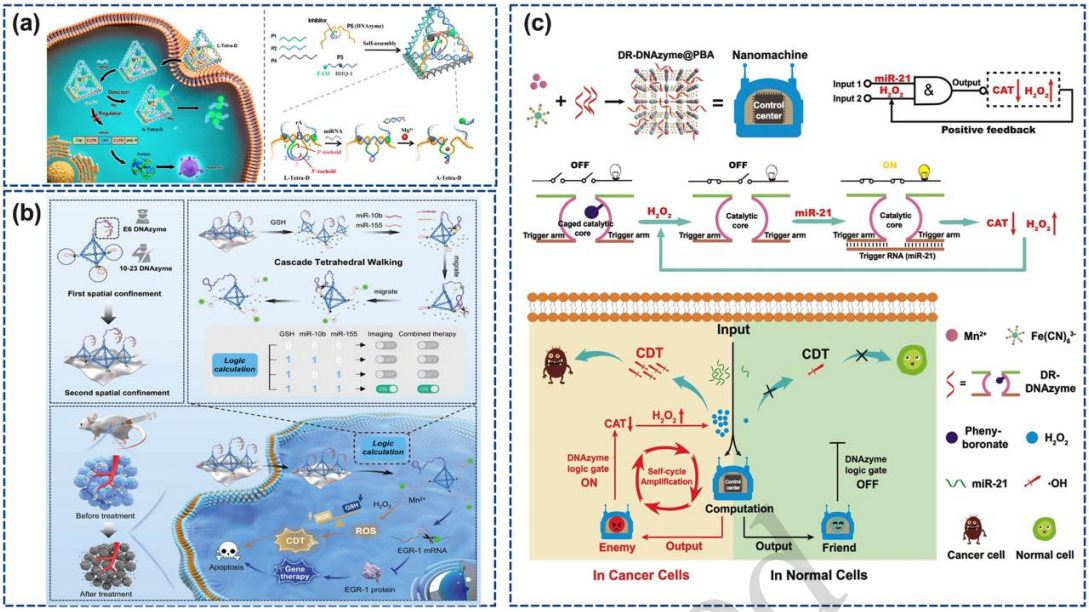

Fig. 3 Engineered Nanoplatforms for DNAzyme Integration. (a) TDN nanomachine enabling miRNA-responsive activation through toehold-mediated strand displacement for precise intracellular sensing and regulation. Reproduced with permission. Copyright 2022, Elsevier (Yu et al., 2022). (b) TriDC platform combining DNAzyme catalysis and cofactor generation for imaging-guided, tumor-selective chemodynamic therapy. Reproduced with permission. Copyright 2025, John Wiley and Sons (S. Zhao et al., 2025). (c) DNAzyme logic nanomachine operating via an AND-gate mechanism, where miR-21 and H2O2 inputs uncage the catalytic core to trigger cell-specific CDT while remaining inactive in normal cells. Reproduced with permission. Copyright 2022, John Wiley and Sons (Z. Wang et al., 2022).

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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 stimulus-responsive disassembly, but may suffer from premature dissolution, less precise structural uniformity, and potential ion-associated toxicity, all of which potentially affecting 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, whereas reproducibility, physiological robustness, and scalable manufacturing remain common bottlenecks requiring further standardization.

Table 1 Comparison of Three Key DNAzyme Nanoplatforms

Nanoplatform	Activation Mechanism	Advantages	Limitations
Metal-Oxide Scaffolds	pH-responsive, Redox-activated	Simple synthesis, Intrinsic cofactor support, Efficient catalytic activation	Premature dissolution, Lack of structural uniformity, Toxicity risks
DNA-Framework	Logic-gated, Substrate-responsive	High programmability, Structural precision, Sequence-specific activation	Nuclease degradation, Complex synthesis, Limited in vivo stability
MOF	pH-triggered, Redox-activated, Co-delivery of multiple agents	High cargo loading capacity, Multifunctional, Potential for co-delivery of drugs/imaging agents	Stability, Batch-to-batch variability, Release kinetics challenges

3.3 Delivery barriers and the limits of stimulus selectivity

Although stimulus-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; Y. Zhao 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 solutions that combine penetrability, cytosolic accessibility, biodegradability, and stricter multi-factor recognition for more reliable *in vivo* catalysis.

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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 tumor-associated macrophages (TAMs) and regulatory T cells (Tregs) (Binnewies et al., 2018; Y. Guo et al., 2024; R. 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 (X. Zhao et al., 2022; Z. Zhao et al., 2025). Additionally, the DNAzyme-mediated reprogramming of metabolic pathways can influence the activity of immunosuppressive populations like M2-like tumor-associated macrophages (TAMs),myeloid-derived suppressor cells (MDSCs) and Tregs (Dong et al., 2023; J. Guo 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. developed a Zn^{2+}-responsive DNAzyme embedded in ZIF-8 (Wu et al., 2022). In acidic TME conditions, Zn^{2+} release was shown to inhibit glycolytic enzymes, depleted nicotinamide adenine dinucleotide^{+} (NAD^{+}), and activate DNAzyme cleavage of GLUT1 mRNA, blocking glucose uptake. The combined effects induced ATP depletion, ROS accumulation and mitochondrial depolarization, collapsing the metabolic reserve of the tumor (Fig. 4a). Furthermore, Yan et al. designed a Ca^{2+}-dependent DNA framework that combined DNAzyme-mediated GLUT1 silencing reduced nicotinamide adenine dinucleotide phosphate (NADPH) and GSH synthesis, while Ca^{2+} overload triggered mitochondrial permeability transition (Yan et al., 2024). 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.

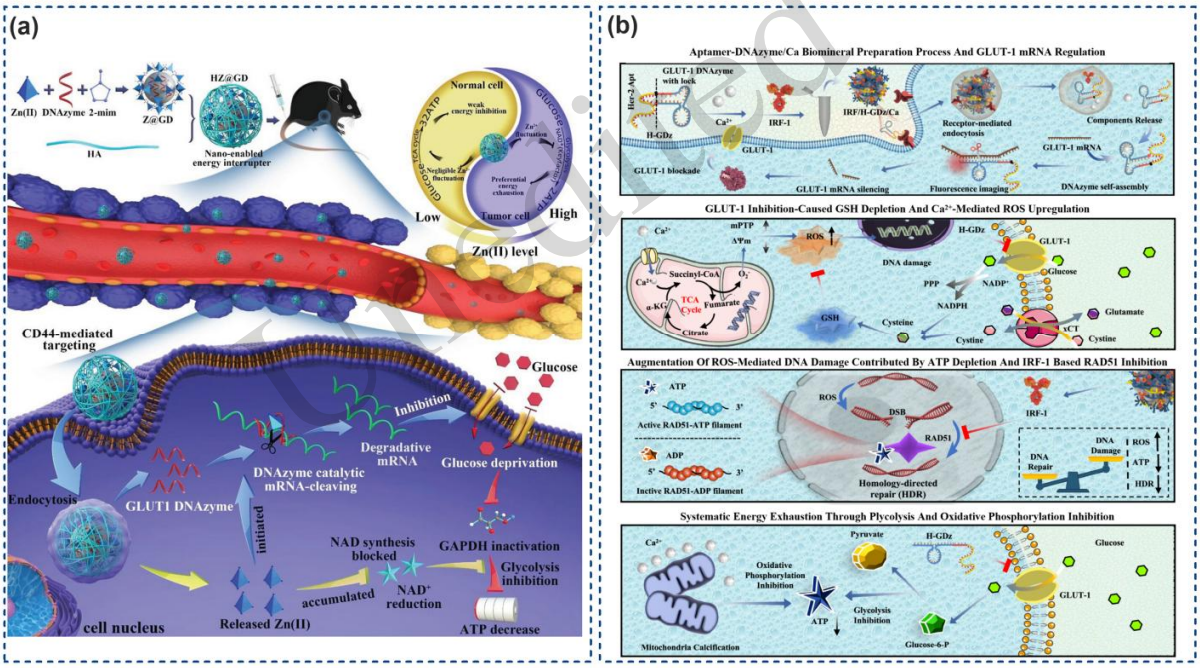

Fig. 4 DNAzyme nanoplatforms for redox modulation and mitochondrial restoration. (a) Schematic of the Zn^{2+}/DNAzyme "energy-interrupter" nanoplatform. Reproduced with permission. Copyright 2021, John Wiley and Sons (Wu et al., 2022) (b) Metal-nucleic-acid frameworks for redox and mitochondrial regulation. GLUT1 suppression disturbs GSH/ROS balance, promotes mitochondrial Ca^{2+} overload and membrane-permeability transition, and reduces ATP production. Reproduced under the terms of the CC BY license. Copyright 2024, Springer Nature (Yan et al., 2024).

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. developed a core-shell nanomedicine (DPCPX@Dz) combining an adenosine A1-receptor inhibitor (DPCPX) with an anti-programmed death-ligand-1 (anti-PD-L1) DNAzyme (J. Guo et al., 2023). While adenosine blockade initiated immunogenic cell death (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. engineered a

MnO2-adjuvant DNAzyme system (H/LDz-M@B) that activated cyclic GMP-AMP synthase-stimulator of interferon genes (cGAS-STING) via a miR-21-initiated hybridization chain reaction and simultaneously silenced PD-L1 through miR-146a-responsive DNAzymes (C. Chen et al., 2023). This dual pathway enhanced IFN-β production, dendritic-cell maturation, and T-cell recruitment (Fig. 5b). Xu et al. introduced a ZnO-Au@mSiO$_2$ radio-immunoenhancer in which X-ray-triggered Zn^{2+} release activated PD-L1-cleaving DNAzymes and the cGAS-STING axis (Xu et al., 2024). This synergy strengthened ICD, boosted IFN-β and TNF-α secretion, and enhanced T-cell infiltration in non-small-cell lung cancer (Fig. 5c). Chen et al. further expanded this concept by developing a NIR-triggered a UCNP-MnO$_2$ nanomachine for the co-delivery of Cas9 RNPs and DNAzymes (C. Chen et al., 2025). Light-induced MnO$_2$ decomposition activated PD-L1 silencing and Clustered regularly interspaced short palindromic repeats (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.

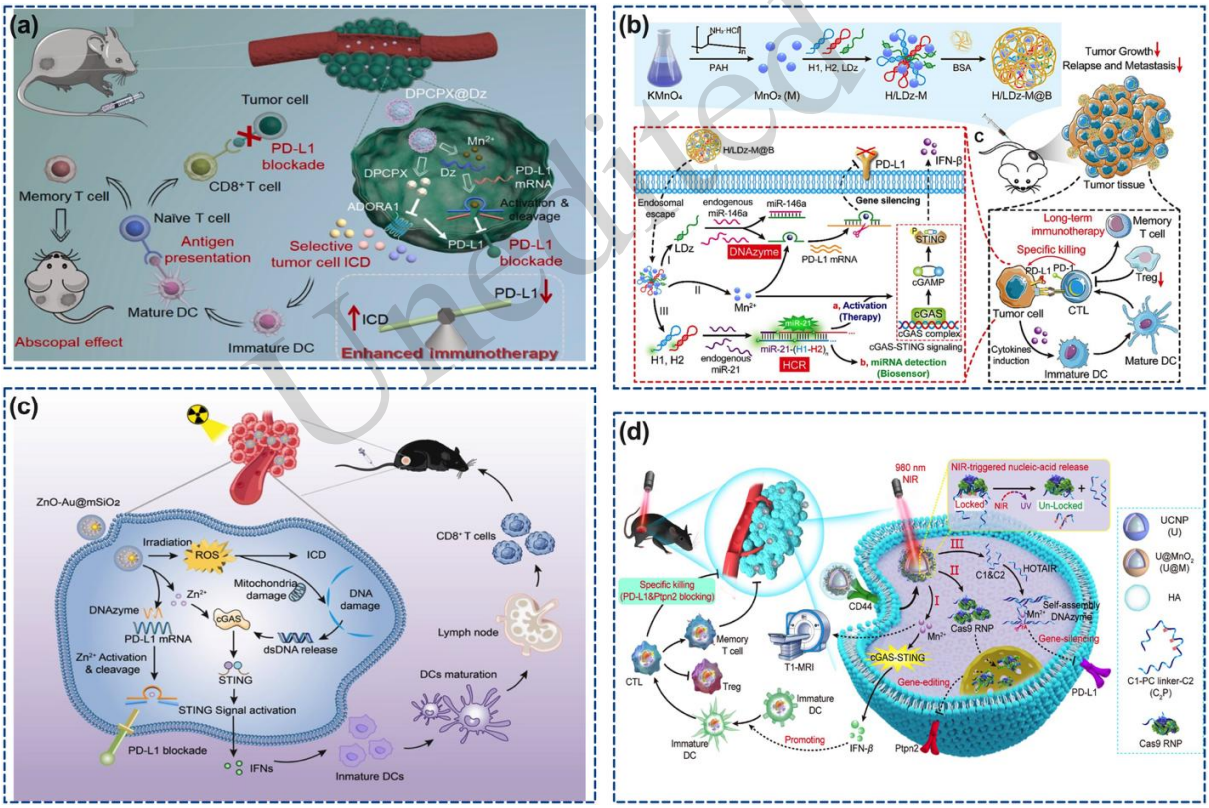

Fig. 5 DNAzyme nanoplatforms for targeted immune modulation. (a) DPCPX@Dz nanoplatform that induces ICD through ADORA1 inhibition while Mn^{2+}-activated PD-L1 DNAzyme down-regulates PD-L1 to enhance T-cell-mediated immunity. Reproduced with permission. Copyright 2023, Elsevier (J. Guo et al., 2023). (b) miRNA-triggered theranostic system that activates cGAS-STING signaling and PD-L1 mRNA cleavage via dual DNAzyme circuits. Reproduced with permission. Copyright 2023, Elsevier (C. Chen et al., 2023). (c) ZnO-Au@mSiO$_2$ nanoparticles enabling X-ray-enhanced ICD and Zn^{2+}-activated DNAzyme/cGAS-STING pathways for amplified immune response. Reproduced under the terms of the CC BY license. Copyright 2024, Springer Nature (Xu et al., 2024). (d) NIR-responsive UCNP-MnO$_2$ nanomachine co-delivering Cas9 RNPs and DNAzymes for light-controlled PD-L1 silencing and immune activation. Reproduced with permission. Copyright 2025, Elsevier (C. Chen et al., 2025).

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 (siRNA) and antisense oligonucleotides (ASOs), and CRISPR-Cas systems, DNAzyme nanocatalysts exhibit distinct mechanistic advantages. While checkpoint inhibitors primarily act through the systemic blockade of immune checkpoint pathways, DNAzymes-based platforms can couple immune checkpoint regulation with direct metabolic remodeling, enabling broader immunometabolic reprogramming (H. Zhang & Zhu, 2026). In contrast to siRNA and ASOs, which depend on endogenous 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 (J. Wang 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, hence DNAzyme systems must still overcome challenges related to delivery, stability, and clinical validation.

Future progress will rely on developing systems-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.

Table 2 Representative programmable DNAzyme nanoplatforms for tumor immunometabolic modulation, featuring the 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	(S. Du 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	(P. Liu 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	(X. Zhao 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	(J. Guo et al., 2024)
ZnO-Au@$mSiO_2$	Zn^{2+}	PD-L1 mRNA	Oxidative stress	Improved radio	X-ray	(Xu et al.,

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			enhancement; ICD; cGAS-STING activation	immunotherapy	dependence; composite safety concerns	2024)
Mn virus-mimicking nanomedicine	Mn^{2+}	miR-19a/PTEN	cGAS-STING activation; STAT3 inhibition; Treg reduction	Anti-metastatic efficacy	Brain-delivery translation uncertainty	(Z. Zhao 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	(X. 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	(J. 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	(C. 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	(C. Chen et al., 2025)

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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 TLR9, in a manner that depends on CpG presentation, sequence context, chemical stabilization, and intracellular trafficking (R. R. Du 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 (J. Wang 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 RNA-cleaving DNAzymes 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 perspective

The integration of DNAzyme catalysis with nanoscale engineering has established a new class of stimulus-responsive nanoplatforms for sensing and modulating tumor immunometabolism. By combining the sequence specificity of RNA-cleaving DNAzymes 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 the 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 minimal off-target effects. The design of hierarchical and logic-gated nanostructures that selectively respond to combinations of endogenous stimuli may further improve conditional selectivity and reduce systemic exposure. In particular, future studies should quantitatively determine whether stimulus-responsive ion release can generate and sustain catalytically effective local cofactor concentrations *in vivo* without inducing 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 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

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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.

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Author contributions

Rongping Luo: literature collection, writing – original draft, and visualization. Zhuojia Tang: literature collection, writing – original draft, and visualization. Mengqi Ding: literature collection and writing – review & editing. 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

The authors that they have no conflicts of interest.

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.

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