

Bifunctional Nanozymes for Synergistic Treatment by Boosting Oxidative Stress and Regulating the Protein Kinase B Pathway

Qi Fang,[#] Xiaojun Zhang,[#] Chong-Bo Ma,^{*,#} Quanyi Liu, Jingyuan Yu, Jiaqi Li, Hao Sun, Jicheng Ma, Hui Wei,^{*} and Yan Du^{*}



Cite This: <https://doi.org/10.1021/acsnano.6c12630>



Read Online

ACCESS |

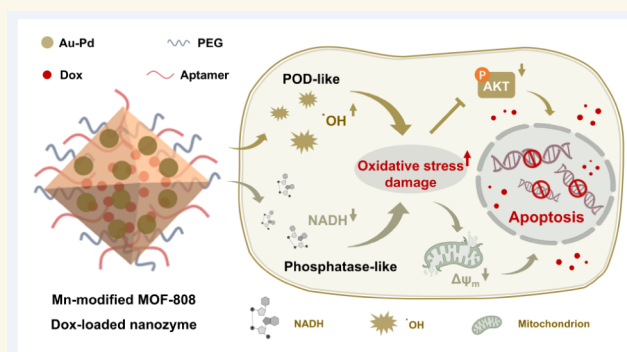
Metrics & More

Article Recommendations

Supporting Information

ABSTRACT: Chemoresistance and dose-limiting toxicity remain major challenges in cancer chemotherapy, largely driven by aberrant activation of the protein kinase B (AKT) survival pathway. Although small-molecule AKT inhibitors can enhance chemosensitivity, their systemic activity frequently disrupts normal metabolic processes and causes severe side effects. Here, we report an “all-in-one” catalytic nanodrug that integrates a dual-enzyme-mimicking nanozyme (GPMM) with doxorubicin (Dox) to overcome these limitations. GPMM simultaneously exhibits peroxidase (POD)-like and phosphatase-like activities, enabling two synergistic biochemical actions: (i) amplification of reactive oxygen species (ROS) through tumor-microenvironment-responsive catalytic conversion of endogenous hydrogen peroxide (H_2O_2), and (ii) depletion of intracellular nicotinamide adenine dinucleotide (NADH) to weaken antioxidant defenses. This dual modulation induces persistent oxidative stress and leads to precise inhibition of AKT phosphorylation, functioning as an effective and safer alternative to conventional AKT inhibitors. As a result, Dox@GPMM markedly enhances chemosensitivity and suppresses tumor growth in nonresistant MCF-7 breast cancer models, while the MUC1 aptamer-functionalized formulation AP-Dox@GPMM effectively reverses Dox resistance in MCF-7-ADR models, without observable systemic toxicity. RNA-sequencing analysis further reveals that the nanozyme regulates key genes involved in metabolism, survival, and AKT-related signaling, thereby reshaping cellular response to chemotherapy. Collectively, this nanozyme-driven redox strategy provides a robust and generalizable approach for boosting chemotherapeutic efficacy while minimizing adverse effects, offering strong translational potential for overcoming resistance across diverse tumor types.

KEYWORDS: nanozyme, synergistic treatment, chemosensitivity, protein kinase B (AKT) pathway, oxidative stress, drug resistance



Chemotherapy remains a cornerstone in the treatment of malignant tumors; however, its inability to discriminate between cancerous and normal cells often leads to severe systemic toxicity and limited efficacy.¹ Enhancing the chemosensitivity of cancer cells can effectively reduce the required therapeutic dose, minimize side effects, and even prevent the emergence of drug resistance.^{2,3} Manipulation of survival-associated signaling pathways has proven to be a promising approach to this end.^{4,5} Among these, the protein kinase B (AKT) signaling axis plays a pivotal role in regulating cell proliferation and survival. Its down-regulation suppresses multiple downstream effectors, thereby enhancing chemotherapy-induced cytotoxicity and genotoxicity.^{6–8} Although small-molecule AKT inhibitors have shown promise in sensitizing cancer cells to chemotherapeutic agents by blocking AKT phosphorylation,^{9–11} their clinical applica-

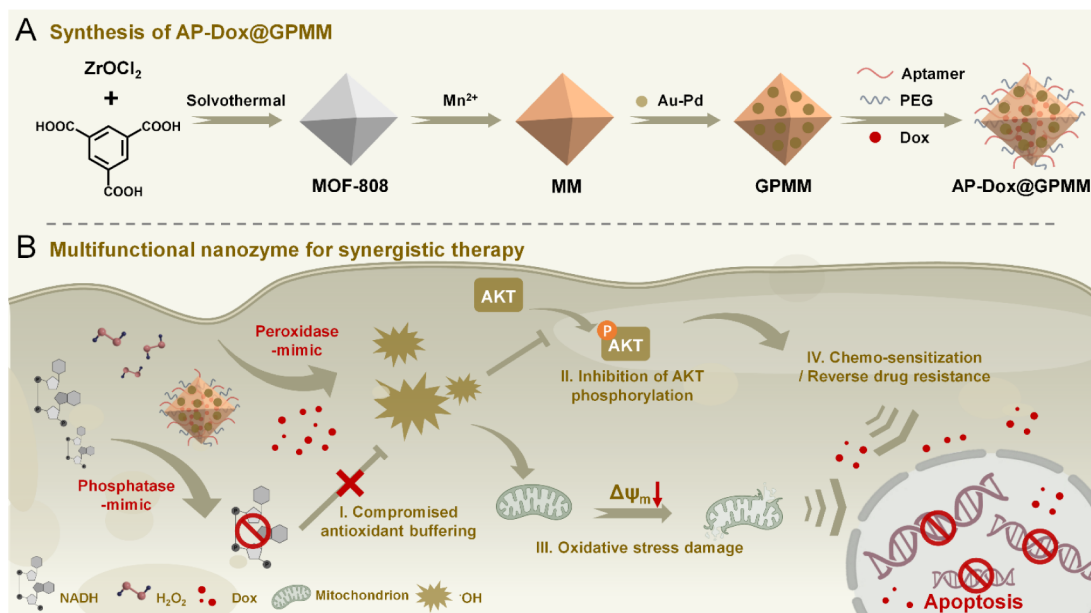
tion remains challenged by limited tumor selectivity and systemic toxicity. Representative clinically advanced AKT inhibitors, such as capivasertib (AZD5363) and ipatasertib (GDC-0068), mainly suppress AKT signaling through direct kinase inhibition. However, the ATP-binding pocket of AKT is highly conserved among human kinases, which complicates isoform-selective targeting and increases the risk of off-target effects.¹² Moreover, systemic AKT inhibition can interfere with

Received: July 3, 2026

Revised: September 18, 2026

Accepted: September 21, 2026

Published: September 29, 2026

Scheme 1. Schematic Illustration of the Synthesis and Antitumor Mechanisms of GPMM-Based Nanozyme Formulationsⁱ

ⁱ(A) Stepwise preparation of Dox@GPMM and AP-Dox@GPMM. MOF-808 was first synthesized *via* a solvothermal reaction, followed by Mn incorporation to obtain MM. Subsequent *in situ* growth of Au–Pd alloy nanoparticles generated GPMM. GPMM was PEGylated with mPEG–OH to obtain PEGylated GPMM, and Dox loading yielded Dox@GPMM. For the targeted resistant-tumor formulation, PEGylated GPMM was functionalized with a thiolated MUC1 aptamer through Au–S bonding to obtain AP-GPMM, followed by Dox loading to yield AP-Dox@GPMM. (B) Proposed synergistic therapeutic mechanism. The POD-like activity of GPMM catalyzes the conversion of endogenous H₂O₂ into $\cdot$ OH, while its phosphatase-like activity depletes intracellular NADH and weakens antioxidant buffering. The resulting redox imbalance suppresses AKT phosphorylation, induces mitochondrial membrane depolarization and oxidative stress damage, and enhances Dox-mediated chemotherapy, ultimately promoting apoptosis and reversing drug resistance.

physiological metabolic signaling in normal tissues and is associated with adverse effects such as diarrhea, rash, hyperglycemia, hypertension, and fatigue.¹² These limitations often restrict dosing intensity and therapeutic windows. Consequently, achieving localized, tumor-specific regulation of AKT activity has emerged as a key challenge in developing safe and effective chemosensitization strategies. Reactive oxygen species (ROS), generated from H₂O₂ decomposition, can initiate apoptosis through oxidative stress and inhibition of the AKT pathway.^{13–15} Accordingly, efficient conversion of H₂O₂ into ROS represents a promising means of simultaneously disrupting redox balance and regulating AKT signaling. In recent years, nanomaterials with intrinsic enzyme-like activities, termed nanozymes, have received extensive attention for such catalytic applications.¹⁶ In particular, nanozymes exhibiting peroxidase (POD)-like activity can catalyze the decomposition of H₂O₂ into highly reactive hydroxyl radicals ($\cdot$ OH), leading to the destruction of cellular redox homeostasis and tumor inhibition.^{17,18} Nevertheless, the ROS generated by most nanozymes remain insufficient within the tumor region,¹⁹ and intracellular antioxidants such as nicotinamide adenine dinucleotide (NADH) can effectively neutralize ROS, diminishing therapeutic efficacy.²⁰ Although auxiliary treatments such as phototherapy or sonodynamic therapy have been explored to overcome these issues, they inevitably increase operational complexity and limit clinical translation.²¹ Therefore, developing a catalytic platform capable of maximizing ROS generation and consumption of endogenous reductants remains a pressing yet challenging goal in tumor therapy.

To achieve efficient redox modulation and enhanced chemosensitivity within tumors, an ideal therapeutic platform should integrate three essential functions: catalytic amplification of ROS to induce oxidative stress, depletion of endogenous NADH to weaken the antioxidant defense system, and co-delivery of chemotherapeutic drugs to realize synergistic antitumor effects. Metal organic frameworks (MOFs) possess high porosity, tunable composition, and inherent catalytic potential, which enable them to act simultaneously as enzyme mimics and versatile carriers for therapeutic agents.²² Previous studies have shown that MOFs can exhibit multiple enzymatic functions, including oxidase²³ and phosphatase²⁴ activities, thereby expanding their biomedical utility in various disease treatments.²⁵ Moreover, MOFs can serve as templates for the *in situ* growth of metal nanoparticles, thereby introducing additional catalytic properties and forming multienzyme-mimetic systems with cooperative effects that enhance therapeutic efficacy.²⁶ Inspired by these characteristics, we selected Zr-based MOF-808 as the scaffold because its robust Zr-oxo framework provides high structural stability, favorable biocompatibility, and intrinsic phosphatase-like activity. We then constructed a multifunctional nanozyme by combining the intrinsic phosphatase-like activity of MOF-808 with *in situ* grown Au–Pd alloy nanoparticles that impart POD-like catalytic properties, as illustrated in Scheme 1A. The resulting hybrid, termed GPMM, simultaneously amplifies ROS generation and depletes intracellular NADH, thereby disrupting redox homeostasis and suppressing the AKT pathway to enhance chemosensitivity while minimizing systemic toxicity. For nonresistant MCF-7 studies, GPMM was modified with mPEG–OH through

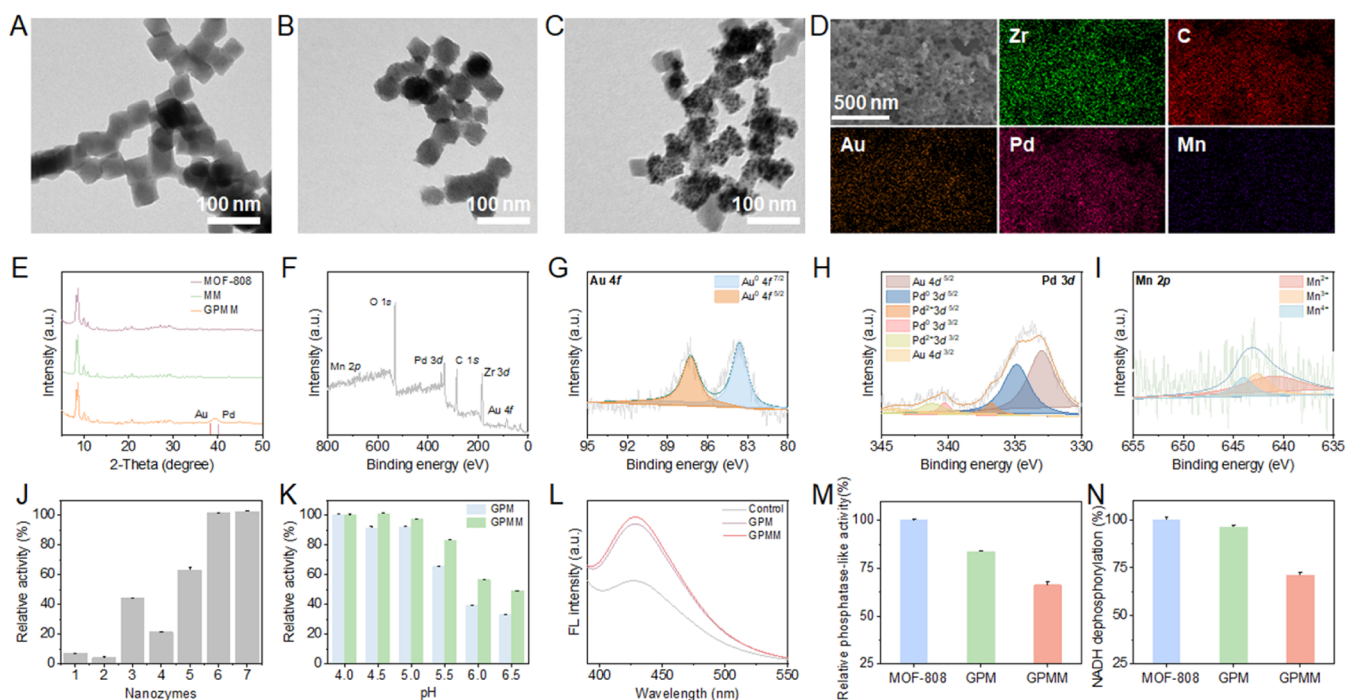


Figure 1. Characterization of nanozymes. (A–C) TEM images of MOF-808, MM, and GPMM. Scale bars: 100 nm. (D) SEM image and EDX elemental mapping of GPMM showing the spatial distribution of Zr, C, Au, Pd, and Mn. Scale bar: 500 nm. (E) XRD patterns of MOF-808, MM, and GPMM. (F–I) Full XPS survey spectrum and high-resolution XPS spectra of GPMM. (J) Relative POD-like activities of different nanozymes (1: MOF-808, 2: MM, 3: GM, 4: PM, 5: GM + PM, 6: GPM, 7: GPMM). Experimental conditions: $50 \mu\text{g mL}^{-1}$ nanozyme, 1 mM TMB, and 5 mM H_2O_2 in 20 mM NaAc-HAc buffer (pH 4.0) at 37°C for 15 min. (K) Relative POD-like activity of GPM and GPMM under different pH values. (L) Fluorescence spectra of $\cdot\text{OH}$ generated using TA assay. Experimental conditions: $100 \mu\text{g mL}^{-1}$ nanozyme, 0.4 mM TA, and 1 mM H_2O_2 in 20 mM NaAc-HAc buffer (pH 6.5) at 37°C for 4 h. (M) Relative phosphatase-like activity of MOF-808, GPM, and GPMM. Experimental conditions: 0.5 mg mL^{-1} nanozyme and 0.1 mM pNPP in 10 mM CHES buffer (pH 9.0) at 37°C for 1 h. (N) NADH dephosphorylation catalyzed by MOF-808, GPM, and GPMM. Experimental conditions: 0.5 mg mL^{-1} nanozyme and 0.1 mM NADH in 2.5 mM Tris-HCl buffer (pH 7.4) at 37°C for 1 h. Data are presented as mean $\pm$ SD ($n = 3$).

noncovalent surface coating and then loaded with doxorubicin (Dox), yielding the nonaptamer-modified formulation Dox@GPM. For Dox-resistant MCF-7-ADR studies, PEGylated GPM was further functionalized with a thiolated MUC1-binding aptamer through Au–S bonding to obtain AP-GPM, and the corresponding Dox-loaded targeted formulation was denoted as AP-Dox@GPM. As depicted in Scheme 1B, the GPM-based nanozyme formulations effectively convert endogenous H_2O_2 into $\cdot\text{OH}$ within the mildly acidic tumor microenvironment (TME), maintain elevated ROS levels through continuous NADH consumption, and inhibit the AKT/NF- κB signaling cascade. Through this dual enzymatic modulation, Dox@GPM and AP-Dox@GPM induce oxidative damage, induce mitochondrial membrane depolarization, and trigger apoptotic cell death in nonresistant and Dox-resistant breast cancer models, respectively. Consequently, Dox@GPM exhibits potent therapeutic efficacy in the nonresistant MCF-7 breast cancer model, whereas AP-Dox@GPM effectively reverses Dox resistance in the MCF-7-ADR model, establishing a robust proof of concept for nanozyme-mediated ROS amplification and AKT pathway regulation in synergistic chemotherapy.

RESULTS/DISCUSSION

Synthesis, Characterization, and Catalytic Performance of Nanozymes

Transmission electron microscopy (TEM) images revealed that all nanozymes maintained a uniform polyhedral

morphology similar to MOF-808, with particle sizes ranging from 30 to 50 nm, and that Au–Pd alloy nanoparticles were successfully generated on the surface of the support (Figure 1A–C and S1). The corresponding energy-dispersive X-ray (EDX) elemental mapping confirmed the homogeneous distribution of C, Zr, Au, Pd, and Mn in GPMM (Figure 1D). X-ray diffraction (XRD) patterns showed that the major diffraction peaks of GPMM and MM coincided well with those of MOF-808, indicating that the crystalline framework of MOF-808 was preserved throughout the synthesis process (Figure 1E). A diffraction peak appearing at approximately 39° was located between the characteristic reflections of Au (PDF No. 04-0784) and Pd (PDF No. 46-1043), confirming the successful formation of a Au–Pd alloy.²⁷ To further analyze the surface composition and valence states, X-ray photoelectron spectroscopy (XPS) measurements were performed. The survey spectrum of GPMM (Figure 1F) verified the coexistence of all expected elements. In the high-resolution Au 4f spectrum (Figure 1G), two distinct peaks at 83.6 and 87.3 eV corresponded to $\text{Au}^0 4f_{7/2}$ and $\text{Au}^0 4f_{5/2}$, respectively. The Pd 3d region (Figure 1H) was deconvoluted into six components, where the binding energies of Pd 3d overlapped with those of Au 4d, and the detected species included $\text{Pd}^0 3d_{5/2}$, $\text{Pd}^0 3d_{3/2}$, $\text{Pd}^{2+} 3d_{5/2}$ and $\text{Pd}^{2+} 3d_{3/2}$, indicating metallic Pd as the dominant form. In addition, the Mn 2p spectrum (Figure 1I) could be resolved into three peaks at 641, 642, and 644 eV, corresponding to Mn^{2+} , Mn^{3+} , and Mn^{4+} species, respectively, confirming the successful incorporation of Mn

into the framework. The coexistence of multivalent Mn states is expected to facilitate redox cycling and promote catalytic activity.²⁸ Furthermore, dynamic light scattering analysis revealed that all samples exhibited hydrodynamic diameters of approximately 100 nm, while the Mn-modified samples (MM and GPMM) showed slightly smaller sizes, likely attributable to structural contraction induced by Mn doping (Figure S2 and Table S1).²⁹

After successful synthesis of the nanozymes, including GPMM, their enzyme-mimicking activities were systematically evaluated to determine the optimal synthesis parameters. The analysis revealed that the optimal metal loading and Mn feed ratio were 5 and 7 wt %, respectively (Figures S3 and S4). In assessing POD-like activity, the Au–Pd bimetallic nanozyme GPM (Au–Pd/MOF-808) exhibited markedly enhanced catalytic performance compared with its monometallic counterparts (GM and PM) or their physical mixture, confirming a clear synergistic interaction between Au and Pd rather than a mere additive effect (Figure 1J).³⁰ Moreover, GPMM retained approximately 50% of its catalytic activity under weakly acidic conditions (pH $\approx$ 6.5), representative of the TME, which was significantly higher than that of GPM (Figure 1K). This improvement was attributed to the presence of multivalent Mn species that facilitated H₂O₂ activation in mildly acidic media.^{31,32} The catalytic reactions of all nanozymes followed typical Michaelis–Menten kinetics toward both H₂O₂ and TMB substrates (Figure S5), and steady-state kinetic analysis confirmed that GPMM possessed higher V_{max} values for both substrates than the other samples (Table S2). To further identify the ROS produced, terephthalic acid (TA) was employed as a fluorescence probe for $\bullet$ OH detection. The generation of 2-hydroxyterephthalic acid (TPA–OH) resulted in a strong emission peak at 435 nm, the intensity of which increased significantly in the presence of nanozymes compared with the control group (Figure 1L). Notably, GPMM produced a stronger fluorescence signal than GPM, confirming its superior POD-like activity and enhanced capability to catalyze the conversion of H₂O₂ into toxic $\bullet$ OH species under tumor-relevant conditions (pH 6.5). To further corroborate ROS generation and examine the role of Mn incorporation, electron paramagnetic resonance (EPR) spectroscopy was performed using DMPO as a spin-trapping agent. The control systems without the complete nanozyme/H₂O₂ combination did not show a comparable characteristic DMPO– $\bullet$ OH signal. In contrast, GPMM produced a markedly stronger DMPO– $\bullet$ OH signal than the Mn-free GPM control in the presence of H₂O₂ (Figure S6), indicating that Mn incorporation enhanced $\bullet$ OH generation during H₂O₂ activation. Together with the mixed Mn²⁺/Mn³⁺/Mn⁴⁺ states identified by XPS, these results support the involvement of reversible Mn valence conversion in facilitating electron transfer and H₂O₂ activation under mildly acidic conditions.^{31,32}

The phosphatase-like activity of the nanozymes was further investigated using p-nitrophenyl phosphate (pNPP) as a model substrate,³³ which is hydrolyzed to the yellow product p-nitrophenol (pNP) displaying a characteristic absorbance peak at 400 nm (Figure 1M and S7). Compared with pristine MOF-808, both GPM and GPMM exhibited reduced phosphatase-like activity, likely due to surface modification by the Au–Pd alloy and Mn incorporation, which partially occupied the active sites and pores of the MOF structure.³⁴ Kinetic studies revealed that the catalytic behavior followed typical Michaelis–

Menten kinetics (Figure S8 and Table S3). To further evaluate the stability of GPMM under serum-containing conditions, the nanozyme was incubated in media containing 5% or 10% FBS for 7 days. In 10% FBS, the apparent hydrodynamic diameter transiently increased to 213.8 nm on day 3 and subsequently decreased to 166.9 nm by day 7, while the polydispersity index remained at or below 0.27. The zeta potential also varied during serum incubation, consistent with changes at the particle–serum interface; however, neither the particle size nor the dispersity showed a progressive increase (Figure S9). Moreover, both the POD-like and phosphatase-like activity-associated absorbance signals remained readily detectable and showed no progressive decrease during the 7-day incubation in 10% FBS (Figure S10). These results indicate that GPMM resisted progressive serum-induced aggregation and retained its dual catalytic function under the tested conditions. Notably, NADH, which contains a phosphate moiety,³⁵ plays a central role in cellular antioxidation and tumor-associated signaling pathways.²⁰ Its consumption weakens the intracellular redox defense system and enhances oxidative stress-induced damage. Therefore, we further examined whether these phosphatase-like nanozymes could catalytically deplete NADH. Upon incubation with MOF-808, GPM, or GPMM, a pronounced decrease in the characteristic NADH absorption at 340 nm was observed, confirming catalytic NADH depletion/dephosphorylation (Figure 1N). Consistent with the pNPP-based phosphatase-like activity results in Figure 1M, GPMM showed a relatively weaker NADH-depleting ability than MOF-808 and GPM, which can be attributed to the partial occupation of MOF active sites and pores by Mn incorporation and Au–Pd alloy deposition. The NADH-consuming capability described above was further verified by a concentration-dependent assay. As the concentrations of MOF-808, GPM, and GPMM increased from 0 to 0.3 mg mL^{−1}, the characteristic NADH absorption band at 340 nm gradually decreased, demonstrating concentration-dependent NADH depletion/dephosphorylation by these nanozymes (Figure S11). Furthermore, the depletion of NADH became more pronounced in the presence of H₂O₂ under acidic conditions (Figure S12), suggesting that both the phosphatase- and POD-like activities of GPMM contribute synergistically to NADH consumption, particularly through the $\bullet$ OH generated during its POD-like catalytic process.

Evaluation of the *In Vitro* Catalytic Performance of Nanozymes

After confirming the enzymatic activities of GPMM, its therapeutic performance *in vitro* was subsequently evaluated. To enhance colloidal stability and reduce nonspecific interactions, PEG was used to coat GPMM, yielding a PEGylated nanozyme with improved biocompatibility, as confirmed by hydrodynamic diameter and zeta-potential analyses (Figures S13 and S14 and Table S4). The cytotoxic effects of the nanozymes were assessed in MCF-7 human breast cancer cells using the methyl thiazolyl tetrazolium (MTT) assay. When cells were incubated with varying concentrations of GPMM, MOF-808, or GPM under simulated TME conditions, characterized by the presence of H₂O₂ and mildly acidic pH (6.5), cell viability changed in a concentration-dependent manner, with GPMM showing the strongest cytotoxicity, GPM showing moderate cytotoxicity, and MOF-808 showing negligible toxicity (Figures S15–S17). In contrast, under neutral pH or in the absence of H₂O₂, all

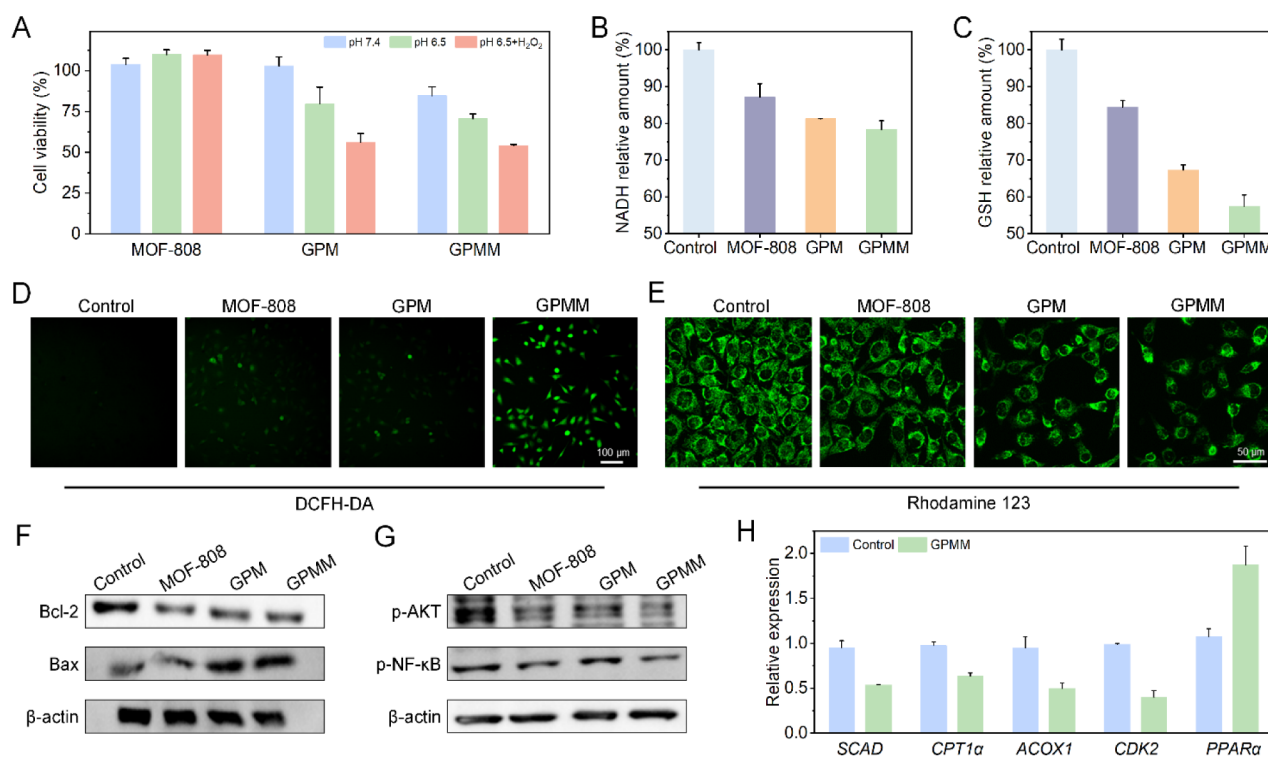


Figure 2. (A) Relative viability of MCF-7 cells after incubation with nanozymes ($100 \mu\text{g mL}^{-1}$) under different conditions for 48 h. (B, C) Intracellular levels of NADH (B) and GSH (C) in MCF-7 cells following treatment with different nanozymes. (D) Fluorescence microscopy images of intracellular ROS assessed using the DCFH-DA probe after pretreatment with nanozymes ($20 \mu\text{g mL}^{-1}$) under pH 6.5 and $100 \mu\text{M}$ H_2O_2 . Scale bar: $100 \mu\text{m}$. (E) Fluorescence images of mitochondrial membrane potential evaluated using rhodamine 123 staining under the same treatment conditions. Scale bar: $50 \mu\text{m}$. (F) Western blot analysis of apoptosis-related proteins Bcl-2 and Bax in MCF-7 cells treated with nanozymes ($20 \mu\text{g mL}^{-1}$). (G) Western blot analysis of p-AKT and p-NF- κB in MCF-7 cells following nanozyme treatment ($20 \mu\text{g mL}^{-1}$). (H) Relative mRNA expression of SCAD, CPT1 α , ACOX1, CDK2, and PPAR α in MCF-7 cells analyzed by real-time quantitative PCR after treatment with nanozymes ($20 \mu\text{g mL}^{-1}$). Data are presented as mean $\pm$ SD ($n = 3$). Statistical significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

nanozymes exhibited minimal cytotoxicity toward MCF-7 cells even at concentrations up to $50 \mu\text{g mL}^{-1}$, demonstrating their excellent biocompatibility under normal physiological conditions. Notably, under TME-simulated conditions, GPMM displayed significantly enhanced cytotoxicity, resulting in approximately 50% cell death at a concentration of $100 \mu\text{g mL}^{-1}$, which was substantially higher than that observed for MOF-808 or GPM (Figure 2A).

To further elucidate the functional impact of the nanozymes, changes in intracellular NADH levels in MCF-7 cells were examined. Following incubation with the different nanozymes, a marked reduction in NADH concentration was observed in all treatment groups, with GPMM producing the most pronounced decrease compared with MOF-808 and GPM (Figure 2B). Although GPMM exhibited relatively weaker phosphatase-like activity, its superior POD-like activity also contributed to NADH consumption, resulting in a more substantial overall decline. This effect was concentration-dependent, with higher GPMM doses leading to greater NADH depletion (Figure S18). Because NADH depletion limits the regeneration of reduced glutathione (GSH), the primary intracellular antioxidant, it consequently promotes the accumulation of ROS.^{36,37} Consistent with this mechanism, cells treated with nanozymes displayed a significant decrease in GSH content relative to the untreated control (Figure 2C), aligning with the NADH depletion trend observed in Figure 2B. To further assess the influence on cellular redox balance,

the oxidizing agent diamide was used to probe changes in antioxidant capacity.³⁷ Cells pretreated with GPMM exhibited markedly reduced viability even at low diamide concentrations, indicating an increased sensitivity to exogenous oxidative stress (Figure S19). The intracellular ROS levels were next quantified using the 2',7'-dichlorofluorescein diacetate (DCFH-DA) probe, which is oxidized by ROS to form fluorescent 2',7'-dichlorofluorescein (DCF). As shown in Figure 2D, nanozyme-treated cells displayed enhanced green fluorescence intensity under simulated TME conditions, confirming elevated ROS accumulation. Among all groups, GPMM induced the strongest fluorescence signal, demonstrating its superior ROS-generating ability. In contrast, negligible fluorescence was detected under neutral conditions (pH 7.4) or in the absence of H_2O_2 (Figure S20), indicating that ROS production by GPMM is TME-dependent, thereby reducing potential damage to normal cells. Furthermore, rhodamine 123 staining was employed to evaluate mitochondrial membrane potential, which can be disrupted by excessive ROS.³⁸ GPMM treatment led to pronounced depolarization of the mitochondrial membrane, evidenced by diminished green fluorescence in the cytoplasm (Figure 2E). Collectively, these findings demonstrate that GPMM effectively perturbs the intracellular redox equilibrium by depleting antioxidants and enhancing ROS generation, thereby compromising mitochondrial integrity and amplifying oxidative damage within tumor cells.

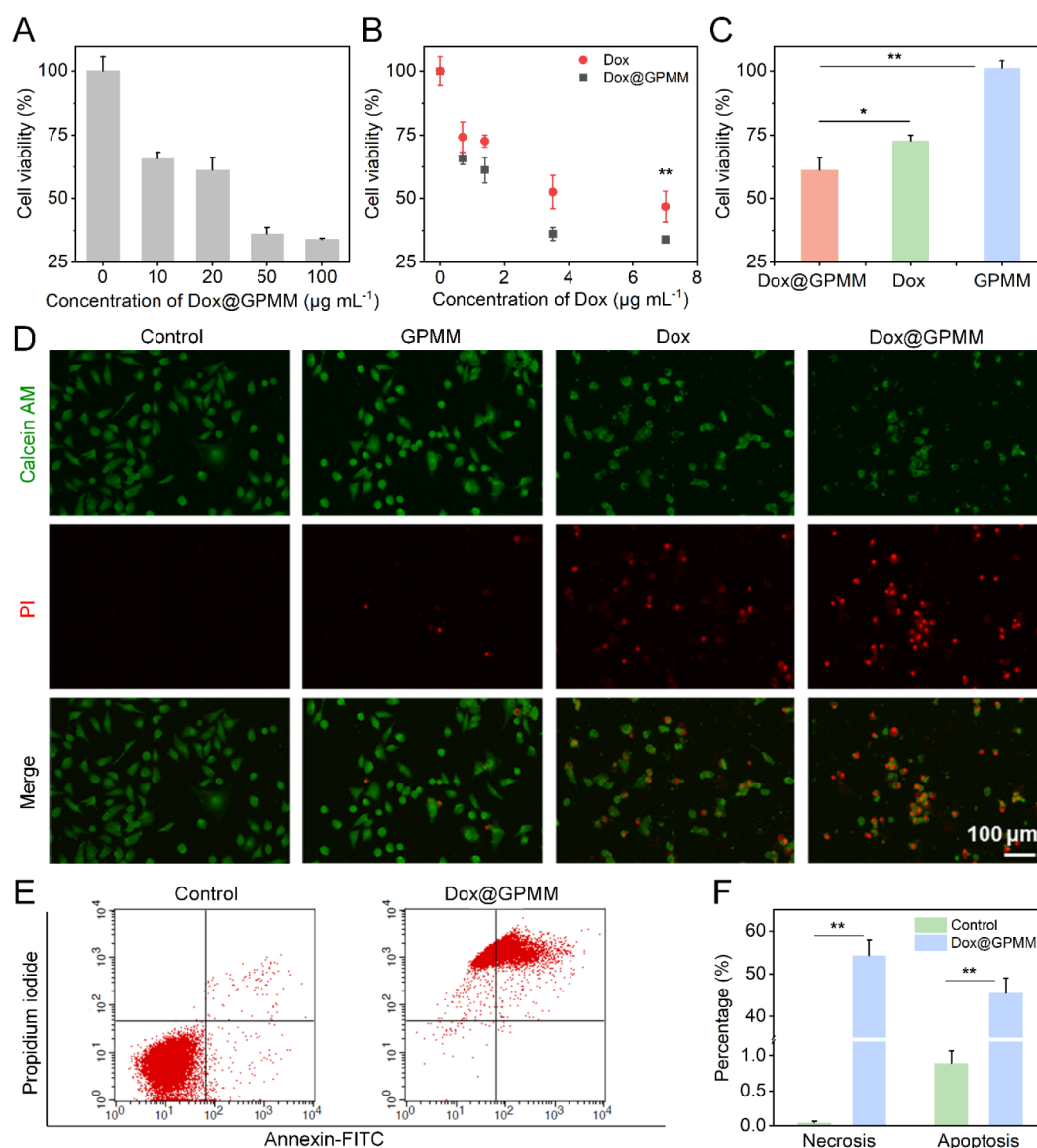


Figure 3. *In vitro* evaluation of the therapeutic efficacy of Dox@GPMM. (A) Relative viability of MCF-7 cells after treatment with various concentrations of Dox@GPMM under pH 6.5 and 100 μ M H₂O₂ for 24 h. (B) Comparison of cell viability following treatment with free Dox or Dox@GPMM at different Dox-equivalent concentrations under pH 6.5 and 100 μ M H₂O₂ for 24 h. (C) Cell viability of MCF-7 cells treated with GPMM (20 μ g mL⁻¹), Dox (1.4 μ g mL⁻¹), or Dox@GPMM (20 μ g mL⁻¹) under the same conditions. (D) Live/dead fluorescence staining of MCF-7 cells in the Control, GPMM (20 μ g mL⁻¹), Dox (1.4 μ g mL⁻¹), and Dox@GPMM (20 μ g mL⁻¹) groups under pH 6.5 and 100 μ M H₂O₂ for 12 h (green: live cells; red: dead cells). (E) Flow cytometric analysis of apoptosis and necrosis in Control and Dox@GPMM-treated MCF-7 cells under pH 6.5 and 100 μ M H₂O₂. (F) Quantification of apoptotic and necrotic cell populations corresponding to panel (E). Data are presented as mean $\pm$ SD (n = 3). Statistical significance: * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

To elucidate the mechanism underlying nanozyme-induced cell death, Western blot analysis was performed to examine the expression levels of apoptosis-related proteins Bcl-2 and Bax, which are associated with antiapoptotic and pro-apoptotic signaling pathways, respectively.³⁹ Overexpression of Bax promotes apoptosis by antagonizing pro-survival proteins, whereas Bcl-2 protects cells from programmed cell death. As shown in Figure 2F, GPMM treatment markedly down-regulated Bcl-2 expression while upregulating Bax expression compared with other groups, indicating the activation of the apoptotic pathway. Given the pivotal role of ROS in modulating the AKT signaling cascade,⁴⁰ we further investigated whether GPMM affected AKT/NF- κ B signaling in MCF-7 cells. Western blot analysis showed that, among the

tested nanozymes, GPMM produced the most pronounced decrease in p-AKT and the downstream phosphorylated nuclear factor κ B (p-NF- κ B) levels (Figure 2G). A concentration-dependent Western blot assay further confirmed that increasing GPMM concentrations progressively suppressed p-AKT and p-NF- κ B expression under pH 6.5 and 100 μ M H₂O₂ (Figure S21). To further examine the relationship between oxidative stress and AKT/NF- κ B signaling, ROSUP and NAC control conditions were included in an additional Western blot experiment (Figure S22). ROSUP treatment decreased p-AKT and p-NF- κ B levels, whereas NAC treatment did not produce a comparable reduction. Together with the enhanced intracellular ROS accumulation and the GPMM-associated inhibition of p-AKT and p-NF- κ B observed

in Figure 2G and S21, these findings are consistent with redox-sensitive modulation of the AKT/NF- κ B signaling axis. Therefore, ROS accumulation is considered a contributing factor to GPMM-mediated pathway suppression, although the present results do not establish it as the sole upstream mechanism. Immunofluorescence imaging further confirmed diminished p-NF- κ B staining intensity across the cytoplasm and nucleus in GPMM-treated cells (Figure S23). As aberrant NF- κ B activation is closely associated with tumorigenesis and reduced chemotherapy efficacy,^{41,42} the concurrent downregulation of p-AKT and p-NF- κ B indicates that GPMM suppresses a critical survival signaling axis linked to cancer cell proliferation and chemoresistance.

To gain deeper insight into the molecular alterations induced by GPMM, we analyzed the expression of metabolism- and cell-cycle-related genes using real-time quantitative PCR (qPCR) (Figure 2H). The results revealed a significant decrease in the mRNA levels of *SCAD*, *CPT1 α* , and *ACO1*, which are key enzymes involved in lipid and fatty acid oxidation,^{43–45} along with a pronounced downregulation of *CDK2*, a central regulator of cell-cycle progression.^{46,47} Additionally, the expression of *PPAR α* was markedly increased following GPMM treatment, consistent with its reported role in promoting apoptosis and enhancing chemosensitivity in cancer cells.⁴⁸ Collectively, these findings demonstrate that GPMM, through its dual enzymatic activities, not only produces ROS in response to the TME but also modulates the AKT/NF- κ B pathway and reprograms metabolic and cell-cycle-related gene expression, thereby promoting oxidative stress-mediated apoptosis and improving chemosensitivity.

In Vitro Evaluation of the Therapeutic Effect of Dox@GPMM

The significant regulatory effect of GPMM on the AKT pathway prompted further investigation into whether this nanzyme could enhance the chemosensitivity of cancer cells and thereby improve therapeutic efficacy. To this end, Dox was encapsulated into GPMM to form Dox@GPMM. The Dox loading content was quantitatively determined by UV–vis spectroscopy at 480 nm using a standard calibration curve, giving a loading capacity of 70 $\mu\text{g mg}^{-1}$, corresponding to 0.07 mg Dox per mg nanzyme carrier. The loading stability was further evaluated by monitoring Dox leakage from Dox@GPMM after 24 h of incubation. Only a slight change in the supernatant absorbance was observed, indicating limited Dox leakage and stable drug retention during the experimental period (Figure S24). After 24 h of incubation, a pronounced reduction in MCF-7 cell viability was observed, declining to approximately 30% at a Dox@GPMM concentration of 100 $\mu\text{g mL}^{-1}$ (Figure 3A). To further verify the chemosensitizing capability of GPMM, the dose-dependent cytotoxic effects of free Dox and Dox@GPMM were compared. Under physiological pH (7.4), both exhibited similar cytotoxicity, reflecting the minimal POD-like catalytic activity of GPMM under neutral conditions (Figure S25). However, under tumor-mimicking conditions (pH 6.5, presence of H_2O_2), Dox@GPMM exhibited markedly enhanced cytotoxicity (Figure 3B). Specifically, 3.5 $\mu\text{g mL}^{-1}$ of Dox in the Dox@GPMM formulation achieved a 65% cell-killing rate, whereas free Dox at double the concentration (7 $\mu\text{g mL}^{-1}$) resulted in only 54% cell death. These results demonstrate that GPMM significantly improves the therapeutic potency of Dox, enabling

dose reduction and minimizing side effects associated with high-dose chemotherapy.

Given the inherent cytotoxicity of GPMM at concentrations above 50 $\mu\text{g mL}^{-1}$, a lower concentration (20 $\mu\text{g mL}^{-1}$) was selected to confirm that the observed improvement originated from enhanced chemosensitivity rather than additive toxicity. As shown in Figure 3C, GPMM alone exhibited negligible cytotoxicity, while the cell-killing efficiencies of free Dox and Dox@GPMM were 28% and 39%, respectively. These findings indicate that Dox@GPMM effectively enhances the chemosensitivity of MCF-7 cells, thereby amplifying the chemotherapeutic efficacy of Dox. Live/dead cell staining further corroborated these results, revealing extensive cell death in the Dox@GPMM-treated group compared with other treatments (Figure 3D). To further evaluate mitochondrial respiratory dysfunction and energy metabolism after combination treatment, mitochondrial complex I activity and intracellular ATP levels were measured in MCF-7 cells treated with Control, Dox, GPMM, or Dox@GPMM. Mitochondrial Complex I is a key component of the electron transport chain responsible for NADH oxidation and is closely associated with cellular oxygen consumption, while intracellular ATP levels provide an indicator of cellular energy status. Compared with the control group, Dox or GPMM alone induced moderate decreases in Complex I activity and ATP levels, whereas Dox@GPMM caused the most pronounced reduction in both parameters (Figure S26). These results indicate that Dox@GPMM synergistically impairs mitochondrial electron transport and energy production, further supporting mitochondrial dysfunction as an important mechanism underlying the enhanced chemotherapeutic effect. Flow cytometric analysis confirmed that Dox@GPMM induced both apoptosis and necrosis, two distinct modes of cell death.⁴⁹ The proportions of apoptotic and necrotic cells reached 45% and 54%, respectively (Figure 3E and 3F). Mechanistically, GPMM-mediated suppression of the AKT/NF- κ B survival pathway promoted apoptosis, while the combination of increased chemosensitivity and Dox exposure further induced necrosis. Collectively, these results demonstrate that GPMM markedly enhances the chemosensitivity of cancer cells, augmenting the antitumor efficacy of Dox through AKT/NF- κ B pathway inhibition and the induction of both apoptotic and necrotic cell death.

To preliminarily explore whether Dox@GPMM treatment was accompanied by molecular changes associated with immunogenic stress, cellular CRT and HMGB1 were examined. Western blot analysis showed treatment-dependent changes in cellular CRT abundance, with the strongest CRT band observed in the Dox@GPMM group (Figure S27).⁵⁰ Immunofluorescence imaging further revealed altered intracellular HMGB1 staining and distribution following GPMM and Dox@GPMM treatments (Figure S28).⁵¹ These observations suggest that GPMM-containing treatments may induce molecular changes associated with ICD; however, they do not by themselves establish DAMP release, downstream immune-cell activation, or systemic antitumor immunity.

In Vivo Evaluation of the Therapeutic Efficacy of Dox@GPMM

Encouraged by the *in vitro* therapeutic performance of GPMM, we further evaluated its efficacy and biosafety *in vivo*. The hematological and histological safety profiles of GPMM were first examined to ensure suitability for animal studies. Hemolysis assays demonstrated excellent blood compatibility,

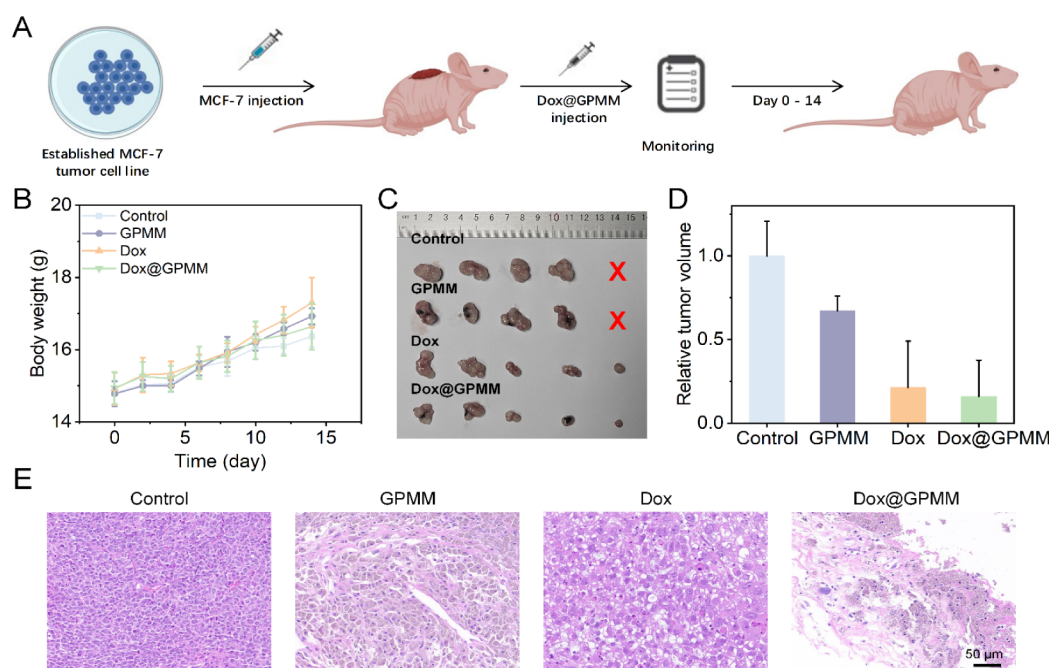


Figure 4. *In vivo* evaluation of the therapeutic efficacy of Dox@GPMM. (A) Schematic illustration of the treatment schedule using a subcutaneous MCF-7 xenograft model in nude mice. (B) Body-weight profiles of mice in the four treatment groups (Control, GPMM, Dox, and Dox@GPMM) over the 14-day study period. (C) Photographs of excised tumors from each treatment group. “X” indicates mice that did not survive the treatment period. (D) Relative tumor volumes of different treatment groups following the single-dose treatment. (E) H&E-stained histological images of tumor tissues collected after treatment. Scale bar: 50 μm. All formulations were administered *via* a single intratumoral injection. The dosage was 25 mg kg^{−1} for GPMM and Dox@GPMM, and 1.75 mg kg^{−1} for free Dox. Data are presented as mean ± SD (*n* = 5).

showing no evident red blood cell lysis even at high GPMM concentrations (Figure S29). Subsequently, a biosafety assessment was conducted by intravenously administering GPMM to Kunming mice. Throughout the observation period, no significant changes in body weight or behavioral abnormalities were detected compared with the control group, and histological analysis of major organs revealed no pathological alterations (Figures S30 and S31). These findings confirmed that GPMM possesses favorable biocompatibility and systemic safety, thereby supporting its feasibility for subsequent *in vivo* therapeutic evaluations.

The *in vivo* antitumor efficacy of the nanozyme system was next evaluated using an orthotopic MCF-7 breast tumor model established in nude mice (Figure 4A). Mice were randomly assigned to four groups: control, GPMM, Dox, and Dox@GPMM. Among the surviving mice, body-weight profiles showed no obvious treatment-related loss during the 14-day observation period (Figure 4B), and end point histological examination of the major organs available for analysis, including the heart, liver, spleen, lung, and kidney, revealed no overt pathological abnormalities (Figure S32), suggesting no evident organ toxicity under the tested conditions. Notably, Dox@GPMM induced a marked suppression of tumor growth, outperforming free Dox as evidenced by tumor photographs and quantitative tumor volume measurements (Figure 4C and 4D). Although GPMM alone exerted moderate antitumor activity, its efficacy was significantly lower than that of the Dox@GPMM combination. Hematoxylin and eosin (H&E) staining further confirmed extensive cellular damage and necrotic regions within tumors treated with Dox@GPMM, in sharp contrast to the control group (Figure 4E). Moreover, immunoblotting analysis revealed a pronounced reduction in

p-AKT levels in tumor tissues from the Dox@GPMM group, consistent with the regulatory effects observed in cultured MCF-7 cells (Figure S33). Collectively, these results demonstrate that Dox@GPMM retains its catalytic activity *in vivo* and provides potent therapeutic efficacy against solid tumors through the synergistic integration of ROS amplification, NADH depletion, and AKT pathway inhibition.

Evaluation of the Therapeutic Efficacy of AP-Dox@GPMM against Drug-Resistant Tumors

A major challenge in chemotherapy is the inevitable development of chemoresistance after prolonged drug exposure, which severely compromises treatment outcomes.⁵² Restoring the chemosensitivity of drug-resistant cancer cells is therefore critical for improving therapeutic efficacy. To extend the applicability of our nanozyme system to drug-resistant tumors, we functionalized GPMM with a MUC1-binding aptamer, denoted as AP, to enhance tumor targeting, yielding AP-GPMM, and investigated its performance in Dox-resistant MCF-7-ADR cells. We first verified that MUC1 aptamer functionalization did not compromise the functional activity of GPMM. Under tumor-mimicking conditions, AP-GPMM produced a concentration-dependent cytotoxic response comparable to that of unmodified GPMM, indicating that the aptamer-modified nanozyme retained the activity of the GPMM catalytic core (Figure 5A). Consistently, fluorescence microscopy showed that both GPMM and AP-GPMM markedly increased intracellular ROS accumulation and reduced mitochondrial membrane potential in MCF-7-ADR cells, indicating that MUC1 aptamer functionalization did not compromise the redox-modulating catalytic activity of GPMM (Figure S34). We further evaluated whether Dox loading could restore the chemosensitivity of MCF-7-ADR cells. Free Dox

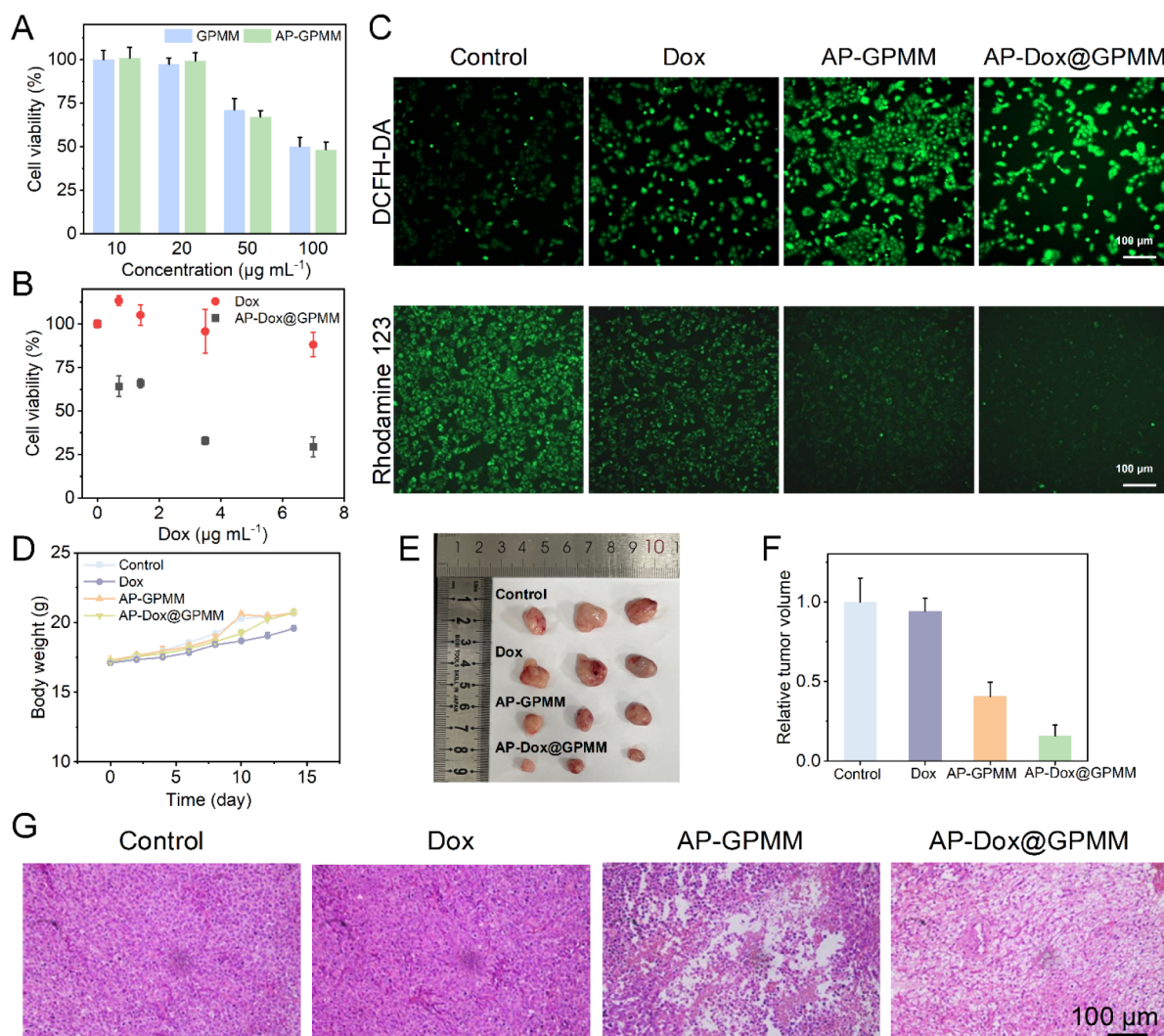


Figure 5. Evaluation of the therapeutic efficacy of AP-Dox@GPMM against MCF-7-ADR drug-resistant cancer cells and tumors. (A) Relative viability of MCF-7-ADR cells after incubation with different concentrations of GPMM or AP-GPMM under pH 6.5 and $100 \mu\text{M H}_2\text{O}_2$ for 24 h. (B) Relative viability of MCF-7-ADR cells treated with free Dox or AP-Dox@GPMM at varying Dox-equivalent concentrations under pH 6.5 and $100 \mu\text{M H}_2\text{O}_2$ for 24 h. (C) Fluorescence microscopy images showing intracellular ROS levels and mitochondrial membrane potential changes in MCF-7-ADR cells treated with Control, Dox, AP-GPMM, or AP-Dox@GPMM under pH 6.5 and $100 \mu\text{M H}_2\text{O}_2$. ROS levels were detected using DCFH-DA staining, and mitochondrial membrane potentials were evaluated using rhodamine 123 staining. Scale bars: $100 \mu\text{m}$. (D) Body-weight curves of MCF-7-ADR tumor-bearing mice receiving Control, Dox, AP-GPMM, or AP-Dox@GPMM over the 14-day treatment period. (E) Photographs of excised tumors from the Control, Dox, AP-GPMM, and AP-Dox@GPMM groups. (F) Relative tumor volumes corresponding to the four treatment groups shown in panel (E). (G) H&E-stained tumor tissue sections from the Control, Dox, AP-GPMM, and AP-Dox@GPMM groups. Scale bar: $100 \mu\text{m}$. All formulations were administered *via* a single intravenous injection. AP-GPMM and AP-Dox@GPMM were administered at 15 mg kg^{-1} , and free Dox was administered at a Dox-equivalent dose of 1.05 mg kg^{-1} . Data are presented as mean $\pm$ SD ($n = 3$).

induced only limited cytotoxicity, confirming the resistant phenotype of MCF-7-ADR cells, whereas AP-Dox@GPMM markedly reduced cell viability at equivalent Dox concentrations (Figure 5B).

To visualize the cellular effects of AP-GPMM and AP-Dox@GPMM on cells more directly, we examined whether AP-GPMM and AP-Dox@GPMM could disrupt redox homeostasis and mitochondrial function in drug-resistant cells using the therapeutically relevant four-group design (Figure 5C). Under tumor-mimicking conditions, free Dox caused only weak ROS accumulation and negligible mitochondrial membrane depolarization, reflecting the reduced sensitivity of MCF-7-ADR cells to Dox. AP-GPMM treatment substantially increased intracellular ROS levels and decreased mitochondrial

membrane potential, confirming that the aptamer-functionalized nanozyme retained its redox-disruptive activity in resistant cells. Notably, AP-Dox@GPMM induced the most pronounced ROS accumulation and mitochondrial depolarization, suggesting that Dox delivery and nanozyme-mediated catalytic stress cooperatively disrupt redox homeostasis in resistant tumor cells. Consistent with this redox-disruptive effect, Western blot analysis showed that AP-GPMM reduced p-AKT expression under tumor-mimicking conditions (Figure S35), further linking nanozyme-induced oxidative stress to suppression of the AKT survival pathway. Moreover, AP-Dox@GPMM exhibited stronger cytotoxicity than AP-GPMM alone under tumor-mimicking conditions (Figure S36), indicating that Dox delivery and nanozyme-mediated oxidative

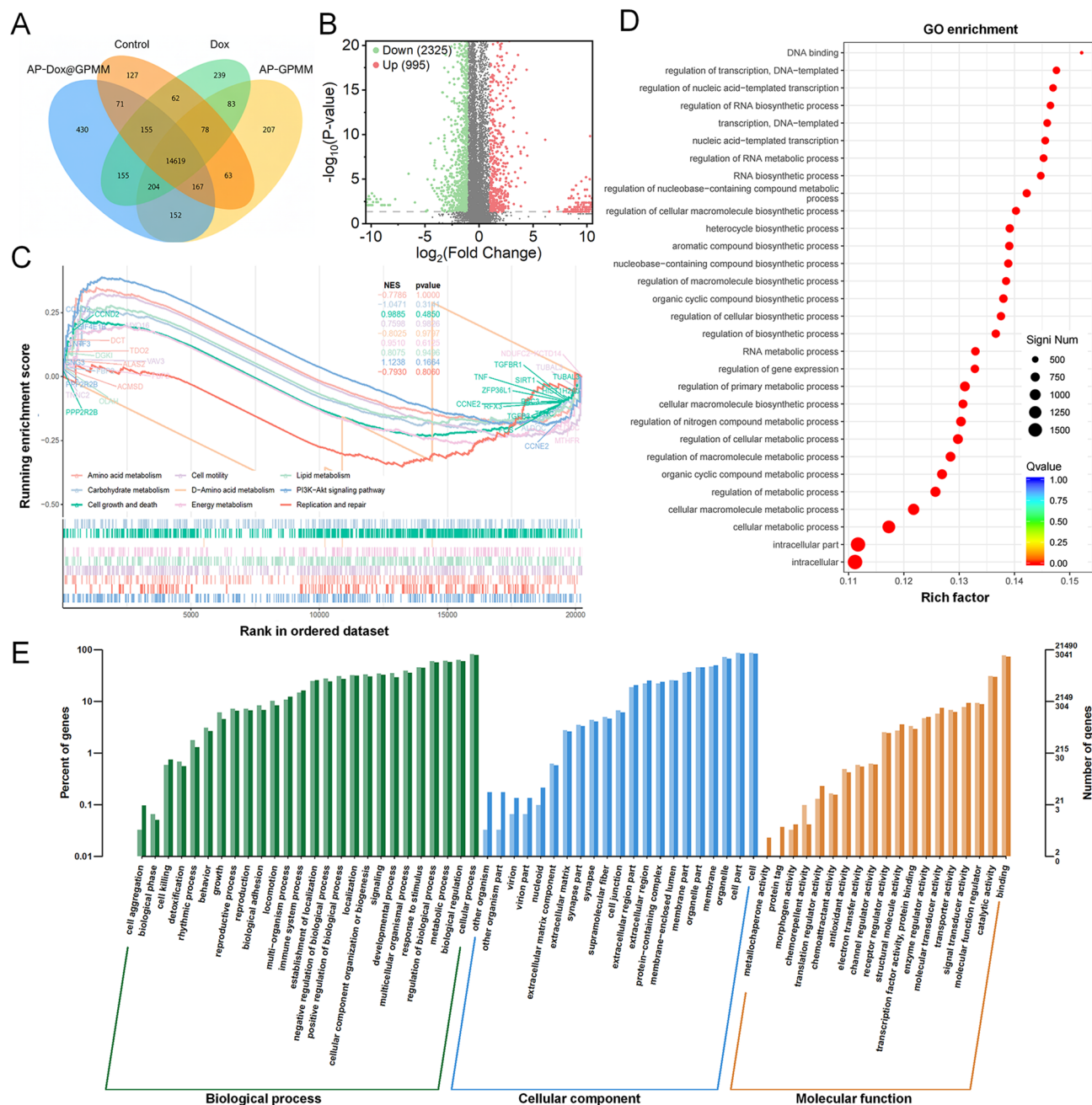


Figure 6. Whole genome RNA expression sequencing analysis. (A) Calculating the intersection of the number of expressed genes with a Venn diagram of control, Dox, AP-GPMM and AP-Dox@GPMM groups. (B) Volcano plot of all gene expression in comparison between Dox and AP-Dox@GPMM groups. (C) GSEA analysis of ranked expression genes by their $\log_2\text{FC}$ between Dox and AP-Dox@GPMM groups. (D) GO terms enrichment of the downregulated genes between Dox and AP-Dox@GPMM groups, top 30 minimum Q value GO terms were shown in the dot plot. (E) GO annotation of differentially expressed genes between Dox and AP-Dox@GPMM groups. Bar graph representing level 2 GO annotation into three categories: biological processes, molecular functions, and cellular components.

stress amplification act cooperatively to overcome Dox resistance. Live/dead staining further corroborated these findings, showing a substantial increase in dead cells and a corresponding decrease in live cells in the AP-Dox@GPMM group (Figure S37). Collectively, these *in vitro* results demonstrate that AP-GPMM induces redox disruption, mitochondrial membrane depolarization, and p-AKT suppression in MCF-7-ADR cells, while AP-Dox@GPMM further enhances Dox-mediated cytotoxicity through concurrent drug delivery and nanzyme-mediated oxidative stress amplification. These findings support the cooperative contribution of POD-

like ROS generation and phosphatase-like NADH depletion to the resensitization of drug-resistant tumor cells to Dox. To determine whether this catalytic synergy is retained *in vivo* in the drug-resistant setting, we next assessed the therapeutic performance of AP-Dox@GPMM in MCF-7-ADR xenograft models. For the *in vivo* therapeutic evaluation, MCF-7-ADR tumor-bearing mice were randomly assigned to four groups: Control, Dox, AP-GPMM, and AP-Dox@GPMM, and each formulation was administered by a single intravenous injection. During the 14-day treatment period, mice in the Control and AP-GPMM groups maintained relatively stable body-weight

profiles, whereas free Dox caused reduced body-weight gain, consistent with the systemic toxicity associated with intravenously delivered Dox (Figure 5D). Consistently, H&E staining of major organs revealed mild hepatic injury in the Dox-treated group, whereas AP-Dox@GPMM caused no obvious histological damage in major organs (Figure S38). By contrast, AP-Dox@GPMM produced the strongest antitumor effect among the four groups, as shown by excised tumor images, tumor-volume measurements, and H&E staining of tumor tissues (Figure 5E–G). As expected for this drug-resistant model, free Dox exhibited negligible therapeutic benefit, with tumor progression comparable to the control group. Notably, AP-Dox@GPMM treatment resulted in no observable tissue damage, and major organs remained histologically normal. The combination of substantial tumor suppression and minimal systemic toxicity highlights the *in vivo* therapeutic synergy between the catalytic nanozyme and Dox, effectively recapitulating the dual redox regulatory effects demonstrated *in vitro*. To further characterize the *in vivo* pharmacological behavior of the PEGylated GPMM-based carrier, we performed ICP-MS analysis to quantify Au concentrations in blood and major organs after intravenous injection. GPMM was detectable in the blood and examined organs at 1 h postinjection, followed by a marked time-dependent decrease from 1 h to 5 days, with only low residual signals remaining at day 5 (Figure S39). These biodistribution and clearance profiles indicate transient systemic distribution followed by progressive clearance from major tissues, thereby reducing the potential risk of long-term tissue retention. Because Dox@GPMM and AP-Dox@GPMM share the same PEGylated GPMM scaffold, this Au-based tracing result reflects the *in vivo* fate of the nanoparticle carrier component of both Dox-loaded formulations and supports the necessity of PEGylation for systemic delivery.

To gain deeper insight into the molecular mechanisms underlying the therapeutic effects of AP-Dox@GPMM on MCF-7-ADR cells, RNA sequencing was performed to identify differentially expressed genes. The Venn diagram identified 127, 239, 207, and 430 group-specific expressed genes in the Control, Dox, AP-GPMM, and AP-Dox@GPMM groups, respectively, together with 14,619 genes shared among all four groups (Figure 6A). Given the strong impact of AP-GPMM on drug resistance and the synergistic enhancement observed with Dox, we focused on comparing AP-Dox@GPMM with free Dox. As shown in Figure 6B, AP-Dox@GPMM induced substantial transcriptional changes, with 995 genes upregulated and 2325 genes downregulated relative to Dox alone. To identify the pathways associated with these regulated genes, Kyoto Encyclopedia of Genes and Genomes (KEGG) and Gene Ontology (GO) enrichment analyses were performed. KEGG pathway classification showed that the differentially expressed genes were distributed across categories related to cellular processes and metabolism, including cell motility, cell growth and death, energy metabolism, and signal transduction (Figure S40). Gene Set Enrichment Analysis (GSEA) included the PI3K–AKT signaling pathway among the examined gene sets and showed changes in the ranked distribution of pathway-associated genes, including PPP2R2B, NTF3, and CCNE2 (Figure 6C).^{53–55} However, the displayed enrichment was not statistically significant; therefore, these transcriptomic findings should be interpreted as exploratory rather than confirmatory evidence of AKT-pathway regulation. GO enrichment (top 30 terms) indicated predominant

involvement in RNA biosynthesis and cellular metabolic processes, both essential for cancer cell survival and apoptosis (Figure 6D).^{56,57} Additionally, GO annotation classified the differentially expressed genes into biological-process categories including cell killing, proliferation, metabolism, and development (Figure 6E).⁵⁸ Parallel transcriptomic comparisons of AP-GPMM versus Control in nonresistant MCF-7 cells and drug-resistant MCF-7-ADR cells (Figures S41–S46) further demonstrated that AP-GPMM modulates gene expression programs governing cell growth and survival in both models. Collectively, these results establish AP-Dox@GPMM as a potent therapeutic platform capable of suppressing diverse tumor types by reprogramming cellular signaling and metabolic pathways. These findings also clarify the mechanistic basis of the nanozyme's therapeutic action: the synergistic interplay between POD- and phosphatase-like activities generates a self-reinforcing loop of oxidative stress. The POD-like conversion of H₂O₂ into ROS initiates inhibition of the AKT pathway, while phosphatase-like NADH depletion weakens the antioxidant defense system, sustaining ROS accumulation. This coordinated redox disruption resensitizes drug-resistant cancer cells to Dox, effectively overcoming chemoresistance without any auxiliary therapeutic intervention. Importantly, the strong agreement between cellular assays and xenograft studies confirms that this nanozyme-driven redox synergy is preserved across biological scales, from *in vitro* tumor cells to *in vivo* drug-resistant tumor models.

CONCLUSIONS

In conclusion, we present a GPMM-based nanozyme-enabled synergistic strategy that simultaneously mitigates chemotherapy-associated toxicity and overcomes acquired drug resistance by coordinating ROS generation and NADH depletion, ultimately achieving precise inhibition of the AKT pathway and enhanced chemosensitivity in MCF-7 tumors using Dox@GPMM and in MCF-7-ADR tumors using AP-Dox@GPMM. In our biological evaluations, GPMM effectively generated ROS under the acidic conditions of the TME and efficiently consumed intracellular NADH, thereby amplifying oxidative stress. Distinct from conventional small-molecule AKT inhibitors that directly block kinase activity and may cause systemic pathway suppression, GPMM regulates AKT phosphorylation indirectly through TME-responsive redox modulation. By combining Mn-enhanced POD-like ROS generation with MOF-808-mediated NADH depletion, GPMM collapses the redox/metabolic buffering capacity that sustains AKT signaling, thereby providing a complementary strategy for AKT pathway regulation with improved tumor selectivity. The comprehensive mechanistic studies presented here elucidate the catalytic behavior, biological functions, and signaling impacts of GPMM, highlighting its environment-responsive and multifunctional characteristics that support its safety and therapeutic potential. Beyond Dox, this redox-modulating nanozyme platform is anticipated to potentiate other chemotherapeutic agents whose efficacy is limited by oxidative imbalance or drug resistance. Considering the central role of AKT signaling in numerous malignancies, the synergistic mechanism demonstrated here may be broadly applicable across a variety of tumor types. Furthermore, the robust consistency between cellular assays and xenograft outcomes confirms that nanozyme-mediated redox modulation is sustained across biological scales, reinforcing the translational promise of this approach. Collectively, this work

establishes a strategy for enhancing chemosensitivity through nanozyme-driven regulation of cellular signaling pathways, offering valuable insights for the future design of effective inhibitors and therapeutic strategies targeting cancer cell survival mechanisms.

METHODS/EXPERIMENTAL

Preparation of the Nanozyme and Drug-Loaded Formulations

MOF-808 was synthesized *via* a solvothermal method using Zr precursors and 1,3,5-benzenetricarboxylic acid as the organic linker, with acetic acid serving as a modulator, following a reported procedure with minor modifications. Typically, $\text{ZrOCl}_2 \cdot 8\text{H}_2\text{O}$ (1.208 g, 3.75 mmol) and 1,3,5-benzenetricarboxylic acid (262.7 mg, 1.25 mmol) were added to a thick-walled glass vessel containing 50 mL DMF and 50 mL acetic acid. The mixture was heated at 130 °C for 24 h. The resulting solid was collected by centrifugation, washed three times with DMF and methanol to remove residual impurities, and dried in a vacuum oven at 40 °C overnight. To prepare MM, 20 mg of MOF-808 was dispersed in 18 mL deionized water containing $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (5.2 mg) and disodium maleate (11.1 mg). The suspension was transferred into Teflon-lined stainless steel autoclaves and heated at 160 °C for 1 h. After cooling, the product was collected by centrifugation, washed three times with deionized water to remove unreacted reagents, and dried in a vacuum oven at 40 °C overnight.

For the preparation of GM, PM, and GPM, MOF-808 was used as the support; for the preparation of GPMM, Mn-decorated MOF-808 (MM) was used as the support. Typically, 10 mg of the corresponding MOF support was fully dispersed in 10 mL of deionized water containing 0.5 mg PVA. For GM and PM, $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ or H_2PdCl_4 solution was added, respectively. For GPM and GPMM, $\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$ and H_2PdCl_4 were added simultaneously. The mixture was stirred vigorously for 30 min, followed by the immediate addition of freshly prepared NaBH_4 solution (0.1 M, $\text{NaBH}_4/\text{total metal} = 4:1$, molar ratio) to initiate reduction. After 10 min, the resulting nanozyme was collected by centrifugation, washed three times with deionized water to remove excess ions and residual reductant, and dried in a vacuum oven at 40 °C overnight. In this nomenclature, GM refers to Au-loaded MOF-808, PM refers to Pd-loaded MOF-808, GPM refers to Au–Pd alloy-loaded MOF-808, and GPMM refers to Au–Pd alloy-loaded Mn-decorated MOF-808.

To improve colloidal stability and biocompatibility, GPMM was coated with methoxy-terminated PEG–OH (mPEG–OH, $M_w \approx 8000$ Da) through a noncovalent surface-coating process. Briefly, GPMM (10 mg) was dispersed in 10 mL of deionized water or PBS, followed by the addition of mPEG–OH (0.1 mg). The mixture was gently stirred at room temperature for 24 h. The mPEG–OH coating was formed through noncovalent adsorption on the GPMM surface, likely involving hydrogen bonding/coordination with exposed surface sites and adsorption onto the PVA-stabilized nanozyme surface. Unbound mPEG–OH was removed by centrifugation and repeated washing with deionized water. The successful PEG coating was supported by hydrodynamic diameter and zeta-potential analyses.

For Dox loading, PEGylated GPMM (10 mg) was dispersed in 10 mL of Dox solution (concentrations ranging from 0 to 0.5 mg mL^{-1}) and stirred at room temperature for 24 h in the dark. The product was collected by centrifugation and washed to remove free Dox, yielding Dox@GPMM. The unencapsulated Dox in the supernatant was quantified by UV–vis spectroscopy at 480 nm using a Dox standard calibration curve. The Dox loading capacity was calculated according to the following equation:

$$\text{Loading capacity (mg mg}^{-1}\text{)} = (\text{m}_{\text{total Dox}} - \text{m}_{\text{free Dox}}) / \text{m}_{\text{GPMM}}$$

To evaluate loading stability, Dox@GPMM was incubated in PBS for 24 h, followed by centrifugation to collect the supernatant. The absorbance of the supernatant at 480 nm was measured to determine Dox leakage.

AP-GPMM was prepared by anchoring a thiolated MUC1 aptamer onto the Au–Pd alloy surface of PEGylated GPMM through Au–S bonding.⁵⁹ Briefly, PEGylated GPMM was prepared as described above and dispersed in a mixture of 10 mM PBS (pH 7.4) and ethanol ($v/v = 1:1$). A thiolated MUC1 aptamer solution was added to the dispersion, and the mixture was gently shaken at 4 °C for 12 h. During this process, the thiol groups of the aptamer formed stable Au–S bonds with the Au–Pd alloy nanoparticles on the GPMM surface. No EDC/NHS coupling chemistry was used in this aptamer-functionalization step. To block residual metal surface sites and reduce nonspecific adsorption, mercaptohexanol (MCH, final concentration: 1 mM) was added and incubated for another 2 h. The resulting AP-GPMM was collected by centrifugation at 10,000 rpm for 10 min, washed three times with deionized water or PBS to remove excess aptamer and MCH, and resuspended in PBS for subsequent use.

AP-Dox@GPMM was prepared by loading Dox into AP-GPMM using the same Dox-loading procedure described above for Dox@GPMM. Briefly, AP-GPMM was dispersed in Dox solution and stirred at room temperature for 24 h in the dark. The product was collected by centrifugation and washed to remove free Dox, yielding AP-Dox@GPMM. The unencapsulated Dox in the supernatant was quantified by UV–vis spectroscopy at 480 nm using a Dox standard calibration curve, and the Dox loading capacity was calculated as described above.

Catalytic Assays

The peroxidase (POD)-like activity of the nanozymes was evaluated by monitoring the oxidation of TMB in the presence of H_2O_2 . In a representative experiment, nanozyme samples (50 $\mu\text{g mL}^{-1}$) were incubated in 500 μL of NaAc–HAc buffer (20 mM, pH 4.0) containing 1 mM TMB and 5 mM H_2O_2 . After incubation at 37 °C for 15 min, the absorbance at 652 nm was recorded. To determine the kinetic parameters toward H_2O_2 , nanozymes (50 $\mu\text{g mL}^{-1}$) were mixed with 500 μL of NaAc–HAc buffer (20 mM, pH 4.0) containing 1 mM TMB and varying concentrations of H_2O_2 (0–2 mM). The reaction proceeded at 37 °C for 15 min, after which the absorbance at 652 nm was measured. The Michaelis–Menten constant (K_m) and maximal velocity (V_{max}) were calculated using the Lineweaver–Burk equation: $1/V = (K_m/V_{\text{max}}) \times (1/C) + 1/V_{\text{max}}$, where V is the initial reaction velocity, C is the H_2O_2 concentration, K_m is the Michaelis constant, and V_{max} is the maximal reaction rate. Kinetic parameters toward TMB were obtained similarly by fixing the concentration of H_2O_2 and varying the TMB concentration (0–1.5 mM).

The generation of hydroxyl radicals ($\cdot\text{OH}$) was evaluated using terephthalic acid (TA) as a fluorescence probe. In a typical experiment, TA, H_2O_2 , and the nanozyme were added to NaAc–HAc buffer (20 mM, pH 6.5) to achieve final concentrations of 0.4 mM, 1 mM, and 100 $\mu\text{g mL}^{-1}$, respectively. The reaction mixture was incubated at 37 °C for 4 h, after which fluorescence spectra were recorded using an excitation wavelength of 315 nm.

Hydroxyl-radical generation was further examined by electron paramagnetic resonance spectroscopy using DMPO as a spin-trapping agent. The reaction mixtures contained 100 mM DMPO, 1 mM H_2O_2 , and 100 $\mu\text{g mL}^{-1}$ nanozyme in 20 mM NaAc–HAc buffer (pH 6.5), with a total volume of approximately 200 μL . Five groups were examined: DMPO alone, DMPO + H_2O_2 , DMPO + GPMM, DMPO + GPM + H_2O_2 , and DMPO + GPMM + H_2O_2 . After mixing at room temperature, the samples were immediately transferred for EPR measurement, and spectral acquisition was initiated within 30–60 s using EPR200-Plus X-band continuous-wave EPR spectrometer (CIQTEK Co., Ltd.).

The phosphatase-like activity of the nanozymes was evaluated using a pNPP hydrolysis assay. In a typical experiment, 400 μL of CHES buffer (10 mM, pH 9.0) containing nanozyme samples (0.5 mg mL^{-1}) and pNPP (0.1 mM) was incubated at 37 °C for 1 h. Following incubation, the absorbance at 400 nm was recorded to quantify the formation of p-nitrophenol.

The catalytic degradation of NADH by the nanozymes was evaluated in a 400 μL reaction system. NADH and the nanozymes were sequentially added to Tris–HCl buffer (2.5 mM, pH 7.4) to

achieve final concentrations of 0.1 mM and 0.5 mg mL⁻¹, respectively. After thorough mixing, the reaction mixture was incubated at 37 °C for 1 h, followed by measurement of absorbance spectra using a NanoDrop spectrophotometer.

To investigate the combined contributions of POD-like and phosphatase-like activities, NADH, H₂O₂, and the nanozyme were added to NaAc-HAc buffer (10 mM, pH 6.5) to obtain final concentrations of 0.1 mM NADH, 1 mM H₂O₂, and 0.2 mg mL⁻¹ nanozyme. After complete mixing, the mixture was incubated at 37 °C for 10 min, and the absorbance spectra were subsequently recorded using a NanoDrop spectrophotometer.

Cellular Assays

MCF-7 human breast cancer cells (FuHeng Biology, Shanghai, China) were cultured in high-glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS; Biological Industries, Israel). The culture medium contained 100 U mL⁻¹ penicillin and 100 U mL⁻¹ streptomycin. All cells were maintained at 37 °C in a humidified incubator with 5% CO₂.

Cell viability was evaluated using the MTT assay. Briefly, 2,000 cells were seeded into each well of a 96-well plate and allowed to adhere. Nanozymes at various concentrations were then added, followed by incubation for 24 or 48 h. After incubation, the MTT assay was performed using a commercial kit (Beijing Solarbio Science and Technology Co., Ltd., China) according to the manufacturer's instructions. The absorbance at 570 nm was recorded to determine cell viability.

Protein concentrations were determined using a BCA protein assay kit (Boster, Wuhan, China). Equal amounts of protein (35 μg per sample) were separated by SDS-PAGE and transferred onto PVDF membranes (Bio-Rad Laboratories, USA). Membranes were incubated with primary antibodies at manufacturer-recommended dilutions, with β-actin used as the loading control. Protein bands were visualized using an enhanced chemiluminescence (ECL) detection kit (Invitrogen, USA). For the ROS-control Western blot experiment shown in Figure S22, MCF-7 cells were seeded in 6-well plates and allowed to adhere overnight. The cells were then treated with control medium, NAC (5 mM), ROSUP (0.5 μg mL⁻¹), or GPMM (20 μg mL⁻¹) under tumor-mimicking conditions (pH 6.5 and 100 μM H₂O₂) for 24 h. After treatment, total proteins were extracted and analyzed by Western blotting using antibodies against p-AKT, p-NF-κB, and β-actin.

MCF-7 cells (1 × 10⁶ cells/well in 6-well plates) were treated with control, GPMM (20 μg mL⁻¹), Dox (1.4 μg mL⁻¹), or Dox@GPMM (20 μg mL⁻¹) for 24 h. Mitochondrial Complex I activity was quantified using a Mitochondrial Complex I Activity Assay Kit. ATP levels were measured with an ATP Assay Kit.

MCF-7 cells were cultured to 70–80% confluence. Untreated control cells and cells treated with Dox@GPMM for 24 h were harvested, washed, stained using an Annexin V-FITC/PI Apoptosis Detection Kit according to the manufacturer's instructions, and analyzed by flow cytometry.

Animal Experiments

Kunming mice and female BALB/c nude mice (6 weeks old) were obtained from the Charles River Animal Company of China. All animal experiments were conducted in accordance with the Guidelines for the Care and Use of Laboratory Animals of the Changchun Institute of Applied Chemistry (CIAC), Chinese Academy of Sciences, and were approved by the Animal Ethics Committee of CIAC (Approval No. 20230001).

Female BALB/c nude mice were used to establish the orthotopic MCF-7 breast tumor model. Briefly, MCF-7 cells were harvested during the logarithmic growth phase and resuspended in PBS. A total of 6 × 10⁶ MCF-7 cells in 200 μL suspension were injected into the subcutaneous tissue of each mouse. When the tumor volume reached approximately 10 mm³, the mice were randomly divided into four groups: Control, GPMM, Dox, and Dox@GPMM (*n* = 5 per group). For the in vivo therapeutic study shown in Figure 4, all formulations were administered via a single in situ injection into the tumor. The Control group received PBS. The GPMM and Dox@GPMM groups

received formulations at a nanozyme dose of 25 mg kg⁻¹, and the free Dox group received Dox at 1.75 mg kg⁻¹. Based on the Dox loading content of 0.07 mg Dox per mg nanozyme carrier, the Dox@GPMM dose corresponded to a Dox-equivalent dose of 1.75 mg kg⁻¹. Body weights and tumor sizes were recorded every 2 days during the 14-day observation period. Tumor volume was calculated according to the following formula:

$$V = 0.5 \times a \times b^2$$

where *a* is the longest tumor diameter and *b* is the shortest tumor diameter. At the end point, surviving mice were euthanized, and tumors and major organs were collected for further analysis.

To evaluate the therapeutic efficacy against Dox-resistant tumors, female BALB/c nude mice were used to establish MCF-7-ADR tumor-bearing models. Briefly, MCF-7-ADR cells were harvested during the logarithmic growth phase and resuspended in PBS. A total of 6 × 10⁶ MCF-7-ADR cells in 200 μL suspension were injected subcutaneously. The mice were randomly divided into four groups: Control, Dox, AP-GPMM, and AP-Dox@GPMM (*n* = 3 per group). Seven days after subcutaneous inoculation of tumor cells, all formulations were administered via a single intravenous injection through the tail vein. The Control group received PBS. AP-GPMM and AP-Dox@GPMM were administered at a nanozyme dose of 15 mg kg⁻¹, and free Dox was administered at a Dox-equivalent dose of 1.05 mg kg⁻¹. Based on the Dox loading content of 0.07 mg Dox per mg nanozyme carrier, the AP-Dox@GPMM dose corresponded to a Dox-equivalent dose of 1.05 mg kg⁻¹. Body weights and tumor sizes were monitored every 2 days during the 14-day observation period. Tumor volume was calculated as described above. At the end point, mice were euthanized, and tumors and major organs were collected for further analysis.

Data Analysis

All data are presented as mean ± SD. Statistical comparisons were performed using two-sided Student's *t* tests or one-way ANOVA, as appropriate. Statistical significance was defined as **p* < 0.05, ***p* < 0.01, ****p* < 0.001, and *****p* < 0.0001.

Additional experimental details, reagent and instrument information, and supporting measurements are provided in the Supporting Information.

■ ASSOCIATED CONTENT

Data Availability Statement

The main data that support the findings of this study are available within the paper and its Supporting Information. Accession codes of transcriptomic data will be available before publication. Further data sets are available from the corresponding author(s) on reasonable request. Source Data are provided with this paper.

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acsnano.6c12630>.

Experimental procedures; nanozyme characterization, catalytic kinetics, serum stability, NADH depletion, cellular assays, drug loading, biosafety, biodistribution, and transcriptomic analyses (Figures S1–S46); particle sizes, kinetic parameters, and qPCR primer sequences (Tables S1–S5) (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Chong-Bo Ma – Key Laboratory of Polyoxometalate and Reticular Material Chemistry of Ministry of Education, National & Local United Engineering Laboratory for Power Batteries, Key Laboratory of Nanobiosensing and

Nanobioanalysis at Universities of Jilin Province, Analysis and Testing Center, Department of Chemistry, Northeast Normal University, Changchun, Jilin 130024, China; Email: macb806@nenu.edu.cn

Hui Wei — School of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing, Jiangsu 210023, China; orcid.org/0000-0003-0870-7142; Email: weihui@nju.edu.cn

Yan Du — State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China; School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China; orcid.org/0000-0003-3197-7204; Email: duyan@ciac.ac.cn

Authors

Qi Fang — Key Laboratory of Polyoxometalate and Reticular Material Chemistry of Ministry of Education, National & Local United Engineering Laboratory for Power Batteries, Key Laboratory of Nanobiosensing and Nanobioanalysis at Universities of Jilin Province, Analysis and Testing Center, Department of Chemistry, Northeast Normal University, Changchun, Jilin 130024, China; State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China; School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China; orcid.org/0009-0003-4562-0908

Xiaojun Zhang — State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China; Department of Physical and Chemical Inspection, Nanguan District Center for Disease Control and Prevention, Changchun, Jilin 130042, China

Quanyi Liu — State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China; School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China; School of Biomedical Engineering, College of Engineering and Applied Sciences, Nanjing National Laboratory of Microstructures, Jiangsu Key Laboratory of Artificial Functional Materials, Nanjing University, Nanjing, Jiangsu 210023, China

Jingyuan Yu — State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China; School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China; orcid.org/0009-0009-9270-6396

Jiaqi Li — State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022, China; School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China; orcid.org/0009-0006-1953-1073

Hao Sun — State Key Laboratory of Electroanalytical Chemistry, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun, Jilin 130022,

China; School of Applied Chemistry and Engineering, University of Science and Technology of China, Hefei, Anhui 230026, China

Jicheng Ma — Key Laboratory of Polyoxometalate and Reticular Material Chemistry of Ministry of Education, National & Local United Engineering Laboratory for Power Batteries, Key Laboratory of Nanobiosensing and Nanobioanalysis at Universities of Jilin Province, Analysis and Testing Center, Department of Chemistry, Northeast Normal University, Changchun, Jilin 130024, China

Complete contact information is available at: <https://pubs.acs.org/10.1021/acsnano.6c12630>

Author Contributions

#Q.F., X.Z., and C.B.M. contributed equally to this work. Y.D. and C.M. directed all experiments and secured funding. Q.F. designed and carried out all the experiments. X.Z. assisted with experiments and discussions. Q.L., J.Y., J.L., and H.S. assisted with experiments. Jicheng Ma conducted the ICP-MS analysis. Y.D., C.M., H.W., and Q.F. wrote and revised the manuscript. All authors approved the final version of the manuscript.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This work was supported by the National Natural Science Foundation of China (Nos. 22322410 and 22574151), the Science and Technology Development Plan Project of Jilin Province (Nos. SKL202402017 and 20250205057GH), and the Major Project of the Changchun State Key Laboratory (2024GZZ13).

REFERENCES

- (1) Letai, A.; de The, H. Conventional Chemotherapy: Millions of Cures, Unresolved Therapeutic Index. *Nat. Rev. Cancer* **2025**, *25*, 209–218.
- (2) Vasan, N.; Baselga, J.; Hyman, D. M. A View on Drug Resistance in Cancer. *Nature* **2019**, *575*, 299–309.
- (3) Lei, Z. N.; Tian, Q.; Teng, Q. X.; Worpel, J. N. D.; Zeng, L.; Pan, Y.; Chen, Z. S. Understanding and Targeting Resistance Mechanisms in Cancer. *MedComm* **2023**, *4*, No. e265.
- (4) Gao, Y.; Chen, D.-L.; Zhou, M.; Zheng, Z.-S.; He, M.-F.; Huang, S.; Liao, X.-Z.; Zhang, J.-X. Cordycepin Enhances the Chemosensitivity of Esophageal Cancer Cells to Cisplatin by Inducing the Activation of AMPK and Suppressing the AKT Signaling Pathway. *Cell Death Dis.* **2020**, *11*, 866.
- (5) Schade, A. E.; Perurena, N.; Yang, Y.; Rodriguez, C. L.; Krishnan, A.; Gardner, A.; Loi, P.; Xu, Y.; Nguyen, V. T. M.; Mastellone, G. M.; Pilla, N. F.; Watanabe, M.; Ota, K.; Davis, R. A.; Mattioli, K.; Xiang, D.; Zoeller, J. J.; Lin, J.-R.; Morganti, S.; Garrido-Castro, A. C.; et al. AKT and EZH2 Inhibitors Kill TNBCs by Hijacking Mechanisms of Involution. *Nature* **2024**, *635*, 755–763.
- (6) Yu, J.; Qin, B.; Wu, F.; Qin, S.; Nowsheen, S.; Shan, S.; Zayas, J.; Pei, H.; Lou, Z.; Wang, L. Regulation of Serine-Threonine Kinase Akt Activation by NAD⁺-Dependent Deacetylase SIRT7. *Cell Rep.* **2017**, *18*, 1229–1240.
- (7) He, H.-N.; Wang, X.; Zheng, X.-L.; Sun, H.; Shi, X.-W.; Zhong, Y.-J.; Huang, B.; Yang, L.; Li, J.-K.; Liao, L.-C.; Zhang, L.; Hu, L.-N.; Lin, Y. Concurrent Blockade of the NF- κ B and Akt Pathways Potently Sensitizes Cancer Cells to Chemotherapeutic-Induced Cytotoxicity. *Cancer Lett.* **2010**, *295*, 38–43.
- (8) Pei, H.; Li, L.; Fridley, B. L.; Jenkins, G. D.; Kalari, K. R.; Lingle, W.; Petersen, G.; Lou, Z.; Wang, L. FKBP51 Affects Cancer Cell

Response to Chemotherapy by Negatively Regulating Akt. *Cancer Cell*. **2009**, *16*, 259–266.

(9) Jabbarzadeh Kaboli, P.; Salimian, F.; Aghapour, S.; Xiang, S.; Zhao, Q.; Li, M.; Wu, X.; Du, F.; Zhao, Y.; Shen, J.; Cho, C. H.; Xiao, Z. Akt-Targeted Therapy As a Promising Strategy to Overcome Drug Resistance in Breast Cancer – A Comprehensive Review from Chemotherapy to Immunotherapy. *Pharmacol. Res.* **2020**, *156*, 104806.

(10) Davies, B. R.; Greenwood, H.; Dudley, P.; Crafter, C.; Yu, D.-H.; Zhang, J.; Li, J.; Gao, B.; Ji, Q.; Maynard, J.; Ricketts, S.-A.; Cross, D.; Cosulich, S.; Chresta, C. C.; Page, K.; Yates, J.; Lane, C.; Watson, R.; Luke, R.; Ogilvie, D.; et al. Preclinical Pharmacology of AZD5363 an Inhibitor of AKT: Pharmacodynamics, Antitumor Activity, and Correlation of Monotherapy Activity with Genetic Background. *Mol. Cancer Ther.* **2012**, *11*, 873–887.

(11) Saura, C.; Roda, D.; Roselló, S.; Oliveira, M.; Macarulla, T.; Pérez-Fidalgo, J. A.; Morales-Barrera, R.; Sanchis-García, J. M.; Musib, L.; Budha, N.; Zhu, J.; Nannini, M.; Chan, W. Y.; Sanabria Bohórquez, S. M.; Meng, R. D.; Lin, K.; Yan, Y.; Patel, P.; Baselga, J.; Tabernero, J.; et al. A First-in-Human Phase I Study of the ATP-Competitive AKT Inhibitor Ipatasertib Demonstrates Robust and Safe Targeting of AKT in Patients with Solid Tumors. *Cancer Discov.* **2017**, *7*, 102–113.

(12) Hua, H.; Zhang, H.; Chen, J.; Wang, J.; Liu, J.; Jiang, Y. Targeting Akt in Cancer for Precision Therapy. *J. Hematol. Oncol.* **2021**, *14*, 128.

(13) Morgan, M. J.; Liu, Z.-G. Crosstalk of Reactive Oxygen Species and NF- κ B Signaling. *Cell Res.* **2011**, *21*, 103–115.

(14) Wen, C.; Wang, H.; Wu, X.; He, L.; Zhou, Q.; Wang, F.; Chen, S.; Huang, L.; Chen, J.; Wang, H.; Ye, W.; Li, W.; Yang, X.; Liu, H.; Peng, J. ROS-Mediated Inactivation of the PI3K/AKT Pathway Is Involved in the Antigastric Cancer Effects of Thioredoxin Reductase-1 Inhibitor Chaetocin. *Cell Death Dis.* **2019**, *10*, 809.

(15) Zhang, G.; He, J.; Ye, X.; Zhu, J.; Hu, X.; Shen, M.; Ma, Y.; Mao, Z.; Song, H.; Chen, F. B-Thujaplicin Induces Autophagic Cell Death, Apoptosis, and Cell Cycle Arrest through ROS-Mediated Akt and P38/ERK MAPK Signaling in Human Hepatocellular Carcinoma. *Cell Death Dis.* **2019**, *10*, 255.

(16) Wei, H.; Gao, L.; Fan, K.; Liu, J.; He, J.; Qu, X.; Dong, S.; Wang, E.; Yan, X. Nanozymes: A Clear Definition with Fuzzy Edges. *Nano Today* **2021**, *40*, 101269.

(17) Meng, X.; Li, D.; Chen, L.; He, H.; Wang, Q.; Hong, C.; He, J.; Gao, X.; Yang, Y.; Jiang, B.; Nie, G.; Yan, X.; Gao, L.; Fan, K. High-Performance Self-Cascade Pyrite Nanozymes for Apoptosis–Ferropoptosis Synergistic Tumor Therapy. *ACS Nano* **2021**, *15*, 5735–5751.

(18) Yang, Q.; Liu, J.; Cai, W.; Liang, X.; Zhuang, Z.; Liao, T.; Zhang, F.; Hu, W.; Liu, P.; Fan, S.; Yu, W.; Jiang, B.; Li, C.; Wang, D.; Xu, Z. Non-Heme Iron Single-Atom Nanozymes As Peroxidase Mimics for Tumor Catalytic Therapy. *Nano Lett.* **2023**, *23*, 8585–8592.

(19) Xu, B.; Cui, Y.; Wang, W.; Li, S.; Lyu, C.; Wang, S.; Bao, W.; Wang, H.; Qin, M.; Liu, Z.; Wei, W.; Liu, H. Immunomodulation-Enhanced Nanozyme-Based Tumor Catalytic Therapy. *Adv. Mater.* **2020**, *32*, 2003563.

(20) Ying, W. NAD⁺/NADH and NADP⁺/NADPH in Cellular Functions and Cell Death: Regulation and Biological Consequences. *Antioxid. Redox Signaling* **2008**, *10*, 179–206.

(21) Liang, S.; Deng, X.; Ma, P. A.; Cheng, Z.; Lin, J. Recent Advances in Nanomaterial-Assisted Combinational Sonodynamic Cancer Therapy. *Adv. Mater.* **2020**, *32*, 2003214.

(22) Wu, M. X.; Yang, Y. W. Metal–Organic Framework (MOF)-Based Drug/Cargo Delivery and Cancer Therapy. *Adv. Mater.* **2017**, *29*, 1606134.

(23) Wu, J.; Wang, Z.; Jin, X.; Zhang, S.; Li, T.; Zhang, Y.; Xing, H.; Yu, Y.; Zhang, H.; Gao, X.; Wei, H. Hammett Relationship in Oxidase-Mimicking Metal–Organic Frameworks Revealed through a Protein-Engineering-Inspired Strategy. *Adv. Mater.* **2021**, *33*, 2005024.

(24) Ma, K.; Cheung, Y. H.; Kirlikovali, K. O.; Xie, H.; Idrees, K. B.; Wang, X.; Islamoglu, T.; Xin, J. H.; Farha, O. K. Fibrous Zr-MOF Nanozyme Aerogels with Macro-Nanoporous Structure for Enhanced Catalytic Hydrolysis of Organophosphate Toxins. *Adv. Mater.* **2024**, *36*, 2300951.

(25) Ma, L.; Jiang, F.; Fan, X.; Wang, L.; He, C.; Zhou, M.; Li, S.; Luo, H.; Cheng, C.; Qiu, L. Metal–Organic-Framework-Engineered Enzyme-Mimetic Catalysts. *Adv. Mater.* **2020**, *32*, 2003065.

(26) Liu, Y.; Cheng, Y.; Zhang, H.; Zhou, M.; Yu, Y.; Lin, S.; Jiang, B.; Zhao, X.; Miao, L.; Wei, C.-W.; Liu, Q.; Lin, Y.-W.; Du, Y.; Butch, C. J.; Wei, H. Integrated Cascade Nanozyme Catalyzes *in Vivo* ROS Scavenging for Anti-Inflammatory Therapy. *Sci. Adv.* **2020**, *6*, No. eabb2695.

(27) Yan, J. M.; Wang, Z. L.; Gu, L.; Li, S. J.; Wang, H. L.; Zheng, W. T.; Jiang, Q. AuPd–MnO_x/MOF–Graphene: An Efficient Catalyst for Hydrogen Production from Formic Acid at Room Temperature. *Adv. Energy Mater.* **2015**, *5*, 1500107.

(28) Yu, L.; Chen, Y.; Wu, M.; Cai, X.; Yao, H.; Zhang, L.; Chen, H.; Shi, J. “Manganese Extraction” Strategy Enables Tumor-Sensitive Biodegradability and Theranostics of Nanoparticles. *J. Am. Chem. Soc.* **2016**, *138*, 9881–9894.

(29) Zimpel, A.; Preiß, T.; Röder, R.; Engelke, H.; Ingrisch, M.; Peller, M.; Rädler, J. O.; Wagner, E.; Bein, T.; Lächelt, U.; Wuttke, S. Imparting Functionality to MOF Nanoparticles by External Surface Selective Covalent Attachment of Polymers. *Chem. Mater.* **2016**, *28*, 3318–3326.

(30) Zhang, X.; Sun, Z.; Jin, R.; Zhu, C.; Zhao, C.; Lin, Y.; Guan, Q.; Cao, L.; Wang, H.; Li, S.; Yu, H.; Liu, X.; Wang, L.; Wei, S.; Li, W.-X.; Lu, J. Conjugated Dual Size Effect of Core-Shell Particles Synergizes Bimetallic Catalysis. *Nat. Commun.* **2023**, *14*, 530.

(31) Watts, R. J.; Sarasa, J.; Loge, F. J.; Teel, A. L. Oxidative and Reductive Pathways in Manganese-Catalyzed Fenton's Reactions. *J. Environ. Eng.* **2005**, *131*, 158–164.

(32) Liu, Y.; Wang, J. Multivalent Metal Catalysts in Fenton/Fenton-Like Oxidation System: A Critical Review. *Chem. Eng. J.* **2023**, *466*, 143147.

(33) Wang, Y.; Yang, L.; Wang, M.; Zhang, J.; Qi, W.; Su, R.; He, Z. Bioinspired Phosphatase-Like Mimic Built from the Self-Assembly of De Novo Designed Helical Short Peptides. *ACS Catal.* **2021**, *11*, 5839–5849.

(34) Ding, X.; Zhao, Z.; Zhang, Y.; Duan, M.; Liu, C.; Xu, Y. Activity Regulating Strategies of Nanozymes for Biomedical Applications. *Small* **2023**, *19*, 2207142.

(35) Bogan, K. L.; Brenner, C. Biochemistry: Niacin/NAD(P), In *Encyclopedia of Biological Chemistry*. Lennarz, W. J.; Lane, M. D., Eds. Academic Press: Waltham, 2013; pp. 172–178.

(36) Xiong, Y.; Su, L.; Ye, F.; Zhao, S. Inhibition of NADP(H) Supply by Highly Active Phosphatase-Like Ceria Nanozymes to Boost Oxidative Stress and Ferroptosis. *Mater. Today Chem* **2022**, *23*, 100672.

(37) Cheng, J.; Li, L.; Jin, D.; Zhang, Y.; Yu, W.; Yu, J.; Zou, J.; Dai, Y.; Zhu, Y.; Liu, M.; Zhang, M.; Sun, Y.; Liu, Y.; Chen, X. A Non-Metal Single Atom Nanozyme for Cutting Off the Energy and Reducing Power of Tumors. *Angew. Chem., Int. Ed.* **2024**, *63*, No. e202319982.

(38) Lu, J.; Song, L.; Feng, S.; Wang, K.; Mao, Y.; Gao, Y.; Zhao, Q.; Wang, S. Nanozyme-Mediated Biocatalysis As a Mitochondrial Oxidative Stress Amplifier for Tumor Nanocatalytic Immunotherapy. *Chem. Eng. J.* **2024**, *481*, 148270.

(39) Han, X.; Li, B.; Wang, W.; Feng, B.; Tang, Q.; Qi, Y.; Zhao, R.; Qiu, W.; Zhao, S.; Pan, Z.; Guo, X.; Du, H.; Qiu, J.; Liu, H.; Li, G.; Xue, H. Cerium Vanadate Nanozyme with pH-Dependent Dual Enzymatic Activity for Glioblastoma Targeted Therapy and Postradiotherapy Damage Protection. *ACS Nano* **2024**, *18*, 19836–19853.

(40) Hasanain, M.; Bhattacharjee, A.; Pandey, P.; Ashraf, R.; Singh, N.; Sharma, S.; Vishwakarma, A. L.; Datta, D.; Mitra, K.; Sarkar, J. A. Solanine Induces ROS-Mediated Autophagy through Activation of

Endoplasmic Reticulum Stress and Inhibition of Akt/mTOR Pathway. *Cell Death Dis.* **2015**, *6*, No. e1860.

(41) Aggarwal, B. B. Nuclear Factor- κ B: the enemy within. *Cancer Cell* **2004**, *6*, 203–208.

(42) Lee, H.; Herrmann, A.; Deng, J.-H.; Kujawski, M.; Niu, G.; Li, Z.; Forman, S.; Jove, R.; Pardoll, D. M.; Yu, H. Persistently Activated Stat3 Maintains Constitutive NF- κ B Activity in Tumors. *Cancer Cell* **2009**, *15*, 283–293.

(43) Li, J.; Huang, Q.; Long, X.; Zhang, J.; Huang, X.; Aa, J.; Yang, H.; Chen, Z.; Xing, J. CD147 Reprograms Fatty Acid Metabolism in Hepatocellular Carcinoma Cells through Akt/mTOR/SREBP1c and P38/PPAR α Pathways. *J. Hepatol.* **2015**, *63*, 1378–1389.

(44) Hu, Y.; Li, J.; Li, X.; Wang, D.; Xiang, R.; Liu, W.; Hou, S.; Zhao, Q.; Yu, X.; Xu, M.; Zhao, D.; Li, T.; Chi, Y.; Yang, J. Hepatocyte-Secreted FAM3D Ameliorates Hepatic Steatosis by Activating FPR1-hnRNP U-GR-SCAD Pathway to Enhance Lipid Oxidation. *Metabolism* **2023**, *146*, 155661.

(45) Li, H.; Chen, J.; Liu, J.; Lai, Y.; Huang, S.; Zheng, L.; Fan, N. CPT2 Downregulation Triggers Stemness and Oxaliplatin Resistance in Colorectal Cancer via Activating the ROS/Wnt/B-Catenin-Induced Glycolytic Metabolism. *Exp. Cell Res.* **2021**, *409*, 112892.

(46) Hochegger, H.; Takeda, S.; Hunt, T. Cyclin-Dependent Kinases and Cell-Cycle Transitions: Does One Fit All? *Nat. Rev. Mol. Cell Biol.* **2008**, *9*, 910–916.

(47) Berthet, C.; Aleem, E.; Coppola, V.; Tessarollo, L.; Kaldis, P. Cdk2 Knockout Mice Are Viable. *Curr. Biol.* **2003**, *13*, 1775–1785.

(48) Zeng, W.; Yin, X.; Jiang, Y.; Jin, L.; Liang, W. PPAR α at the Crossroad of Metabolic–Immune Regulation in Cancer. *FEBS J.* **2022**, *289*, 7726–7739.

(49) Kanduc, D.; Mittelman, A.; Serpico, R.; Sinigaglia, E.; Sinha, A.; Natale, C.; Santacroce, R.; Di Corcia, M.; Lucchese, A.; Dini, L.; Pani, P.; Santacroce, S.; Simone, S.; Bucci, R.; Farber, E. Cell Death: Apoptosis versus Necrosis (Review). *Int. J. Oncol.* **2002**, *21*, 165–170.

(50) Yan, Z.; Bai, Y.; Zhang, S.; Kong, L.; Wang, Y.; Sun, H.; Li, Y.; Qiu, L.; Zhang, R.; Jiang, P.; Zhao, D.; Chen, Z.; Li, Y.; Pang, H.; Wang, J. Quasi Fe MIL-53 Nanozyme Inducing Ferroptosis and Immunogenic Cell Death for Cancer Immunotherapy. *Nat. Commun.* **2025**, *16*, 2290.

(51) Yang, H.; Xu, G.; Li, F.; Guo, G.; Yan, P.; Chen, Y.; Chen, Y.; Sun, W.; Song, W.; Zhong, W. Multi-Enzyme Nanoparticles As Efficient Pyroptosis and Immunogenic Cell Death Inducers for Cancer Immunotherapy. *Adv. Sci.* **2024**, *11*, 2408729.

(52) Sajid, A.; Rahman, H.; Ambudkar, S. V. Advances in the Structure, Mechanism and Targeting of Chemoresistance-Linked ABC Transporters. *Nat. Rev. Cancer* **2023**, *23*, 762–779.

(53) Tormo, E.; Adam-Artigues, A.; Ballester, S.; Pineda, B.; Zazo, S.; González-Alonso, P.; Albanell, J.; Rovira, A.; Rojo, F.; Lluch, A.; Eroles, P. The Role of miR-26a and miR-30b in HER2+ Breast Cancer Trastuzumab Resistance and Regulation of the CCNE2 Gene. *Sci. Rep.* **2017**, *7*, 41309.

(54) Roška, J.; Wachsmannová, L.; Hurbanová, L.; Šestáková, Z.; Mueller, T.; Jurkovičová, D.; Chovanec, M. Differential Gene Expression in Cisplatin-Resistant and -Sensitive Testicular Germ Cell Tumor Cell Lines. *Oncotarget* **2020**, *11*, 4735–4753.

(55) Bao, Y.; Oguz, G.; Lee, W. C.; Lee, P. L.; Ghosh, K.; Li, J.; Wang, P.; Lobie, P. E.; Ehmsen, S.; Ditzel, H. J.; Wong, A.; Tan, E. Y.; Lee, S. C.; Yu, Q. EZH2-Mediated PP2A Inactivation Confers Resistance to HER2-Targeted Breast Cancer Therapy. *Nat. Commun.* **2020**, *11*, 5878.

(56) Martínez-Reyes, I.; Chandel, N. S. Cancer Metabolism: Looking Forward. *Nat. Rev. Cancer* **2021**, *21*, 669–680.

(57) López, J.; Blanco, S. Exploring the Role of Ribosomal RNA Modifications in Cancer. *Curr. Opin. Genet. Dev.* **2024**, *86*, 102204.

(58) Kuperstein, I.; Bonnet, E.; Nguyen, H. A.; Cohen, D.; Viara, E.; Grieco, L.; Fourquet, S.; Calzone, L.; Russo, C.; Kondratova, M.; Dutreix, M.; Barillot, E.; Zinovyev, A. Atlas of Cancer Signalling Network: A Systems Biology Resource for Integrative Analysis of Cancer Data with Google Maps. *Oncogenesis* **2015**, *4*, No. e160.

(59) Hao, Y.; Li, Y.; Song, L.; Deng, Z. Flash Synthesis of Spherical Nucleic Acids with Record DNA Density. *J. Am. Chem. Soc.* **2021**, *143*, 3065–3069.