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Nanozymes regulated by nitrogen element: Mechanism, design, and application

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ABSTRACT

Nanozymes, a category of nanomaterials endowed with enzyme-mimicking capabilities, have exhibited considerable potential across diverse application domains. This comprehensive review delves into the intricacies of regulating nanozymes through N elements, elucidating the mechanisms governing N element control in the design and application of these nanomaterials. The initial sections introduce the foundational background and significance of nanozymes. Subsequent exploration delves into the detailed discussion of N element regulation mechanisms on nanozymes, encompassing N vacancies, N doping, N coordination, and nitride. These regulatory pathways play an instrumental role in fine-tuning the catalytic activity and specificity of nanozymes. The review further scrutinizes practical applications of N element regulation on nanozymes, spanning sensing detection, infection therapy, tumor therapy, and pollutant degradation. In conclusion, it succinctly summarizes the current research findings and proposes future directions for development. This thorough investigation into the regulation of nanozymes by N elements anticipates precise control over their performance, thereby advancing the extensive utilization of nanozymes in the realms of biomedical and environmental applications.

1. Introduction

The emergence of enzyme engineering stems from the urgent demand for enzymes in large quantities, aiming to enhance enzymatic efficiency through various technological means to address challenges such as high costs, poor stability, low efficiency, and limited applicability in industrial production [1]. Despite the broad application of enzyme engineering technologies in facilitating the use of natural enzymes, fundamental issues in their industrial application remain unresolved. Out of over 8300 types of natural enzymes, only about 2% can be produced on a large scale, failing to meet the ever-growing demand. In the mid-20th century, chemists proposed the concept of artificial enzymes, intending to utilize organic small molecules like cyclodextrins and principles of host-guest chemistry to study the chemical essence of enzyme catalysis, and

synthesize mimetic enzymes mimicking the catalytic active center structures and mechanisms of natural enzymes. However, the conventional structures of mimetic enzymes are constrained, making it difficult to fully replicate the fine structures of natural enzymes, and most mimetic enzymes' catalytic activities cannot rival those of natural enzymes. Consequently, scientists have been continuously devoted to exploring artificial mimetic enzymes with high biological catalytic activity. With the development of nanoscience and technology, nanozymes have emerged as a new type of material, revealing unique nanoscale effects, namely, nano materials embodying enzymatic catalytic characteristics [2] (see Scheme 1).

In the realm of emerging materials, nanozymes stand out as a fusion of nanomaterial physicochemical attributes and the catalytic prowess inherent in enzyme-like structures, presenting a dual proficiency akin to

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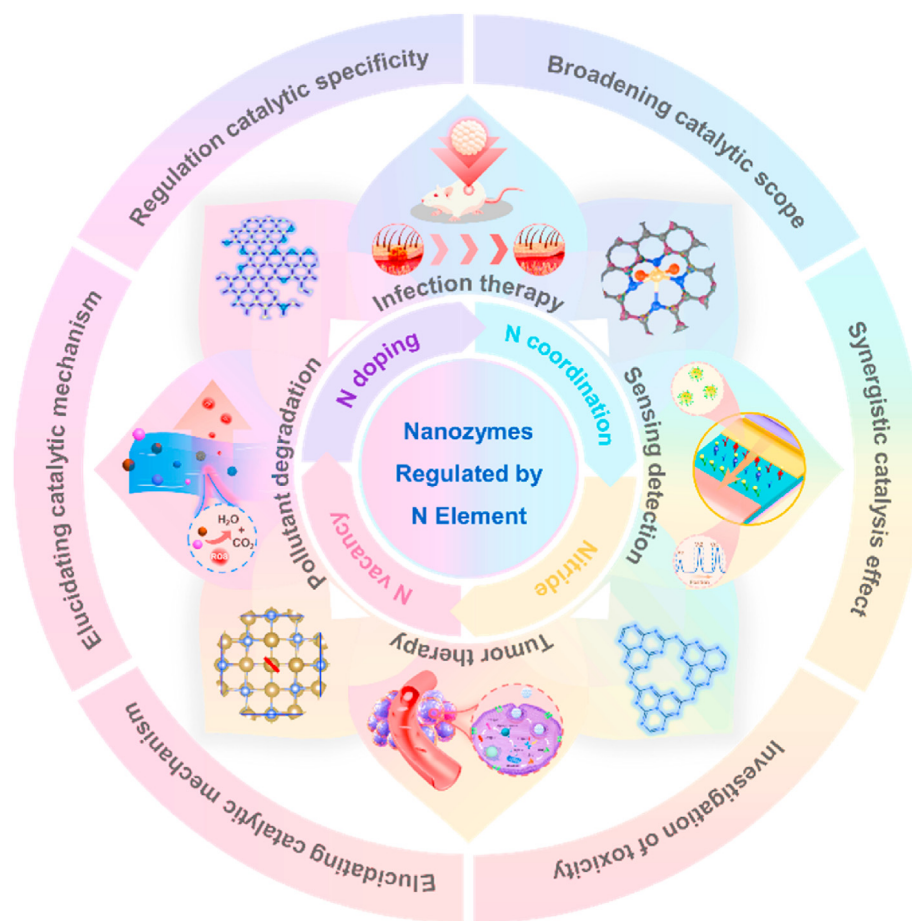
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Scheme 1. Schematic diagram of the nanozymes regulated by N element: mechanism, design, and application.

both natural and artificial enzymes [3–5]. The intricate nanostructure not only endows nanozymes with remarkably efficient catalytic functions but also bestows heightened stability when compared to their natural counterparts, thereby facilitating the prospect of scalable production [6–8]. Moreover, the distinctive multi-enzyme activity exhibited by nanozymes offers a repository of functional molecules, paving the way for the development of cost-effective, stable, and innovative catalytic cascade reactions [9–12]. Recognized by International Union of Pure and Applied Chemistry as one of the foremost innovations in chemistry for 2022, nanozymes epitomize a cross-disciplinary amalgamation [13]. As research delves into the catalytic mechanisms of nanozymes, applications have progressively broadened from initial detection methodologies to encompass diverse domains such as disease treatment, sustainable synthesis practices, novel energy solutions, and effective environmental management [14–17].

A myriad of nanozymes exists, showcasing diverse compositions and structures [18,19]. A meticulous examination of the intricate interplay between structure and activity is imperative for the dependable engineering of nanozymes [20]. In recent times, the escalating volume of nanozyme research has unveiled certain factors wielding profound influence over nanozyme performance [21,22]. These revelations have been strategically harnessed in the rational design of nanozymes. Drawing from shared structure-activity relationships between nanozymes and their natural counterparts, successful strategies have emerged for crafting nanozymes endowed with catalytic microenvironments and active sites akin to those found in natural enzymes [23]. This approach has demonstrably heightened the activity and selectivity of nanozymes [24]. Furthermore, recent strides in single-atom material development techniques have given rise to a cohort of SAzymes, distinguished by their unique catalytic sites [25,26]. Astonishingly, some of these nanozymes

surpass the catalytic prowess of natural enzymes with analogous activity. Concurrently, progress in cutting-edge technologies like big data analysis, machine learning, and theoretical computation has unlocked novel prospects for the high-throughput simulation, design, and optimization of nanozymes [27].

To date, nanomaterials exhibit a wide range of enzyme-mimicking activities, spanning various types including peroxygenase, catalase, superoxide dismutase, oxidase, glucose oxidase, glutathione peroxidase, protease, esterase, and nucleases [28]. Among these, peroxidase (POD)-like nanozymes stand out prominently. These nanozymes, functioning as bifunctional catalysts, can generate a large number of oxidative radicals by catalyzing the hydrogen acceptor in the presence of the first substrate (e.g., H_2O_2), thereby rapidly oxidizing the hydrogen donor of the second reaction substrate. Similarly, catalase-like nanozymes can decompose H_2O_2 into water and oxygen, generating bubbles during the reaction, akin to natural catalases. Oxidase-like nanozymes, on the other hand, utilize oxygen as the hydrogen acceptor to directly oxidize the hydrogen donor substrate without the need for additional compounds like H_2O_2 . Superoxide dismutase (SOD)-like nanozymes, resembling SOD, convert superoxide anions into O_2 and H_2O_2 , exhibiting anti-inflammatory and antioxidative effects. Furthermore, protease-like nanozymes hydrolyze peptide bonds in proteins, breaking them down into peptides or amino acids, while nucleases can cleave phosphodiester bonds in nucleic acid chains, hydrolyzing DNA or RNA into nucleotides [29]. In recent years, with the continual expansion of catalytic types and the sustained enhancement of catalytic activity, research into nanozyme applications has become increasingly extensive and profound. Currently, the application of nanozymes is primarily focused on the biomedical field, including disease diagnosis, biochemical detection and sensing, anticancer therapies, antimicrobial treatments, anti-inflammatory

interventions, and antioxidative strategies. Additionally, some nanozymes have been applied in areas such as wastewater treatment, chemical synthesis, and decontamination in chemical defense [30].

The catalytic efficacy of nanozymes is subject to regulation by a myriad of factors, with the compositional framework of materials assuming a pivotal role in orchestrating this regulatory ballet [31,32]. Categorized based on material composition, nanozymes find themselves distinguished as carbon nanozymes, metal oxide nanozymes, noble metal nanozymes, and an array of other nanozyme varieties [33,34]. Each variant of nanozyme, through deliberate compositional adjustments, exhibits a distinctive ability to selectively modulate the catalytic prowess inherent in its active sites, thereby enabling a meticulously targeted governance of catalytic activities. N, as an element extravagantly abundant, assumes a central role in the regulation of nanozyme structures, notably pertaining to N vacancies, N doping, N coordination, and nitrides [35–37]. The dominion of N extends beyond its influence on electronic configurations within N vacancies, exerting a profound impact on the overarching conformation of nanozymes. The introduction of N doping not only amplifies material conductivity but also finely regulates the catalytic functions of nanozymes, establishing an elevated catalytic milieu. N coordination, an indispensable factor shaping material characteristics, affords precise manipulation of nanozyme structures, thereby refining their efficacy and selectivity in catalytic processes. Nitrides, as an embodiment of N within materials, showcase distinctive electronic traits and structural attributes. The incorporation of nitrides not only broadens the application spectrum of nanozymes but also assumes a significant role in catalyst design within the realm of biomedical research. This holistic comprehension contributes to a profound understanding of the catalytic aptitude inherent in nanozymes, unfurling novel avenues for their precise application across diverse domains.

In 2018, Wei et al. unveiled a groundbreaking discovery

demonstrating that N-doped reduced graphene oxide (N-rGO) profoundly amplifies the catalytic prowess of peroxidase (POD)-like activity [38]. This enhancement arises from the facilitation of oxygen radical formation in proximity to N atoms, which, in turn, are stabilized by the lone pair electrons of N. N-doping can increase the POD-like activity of N-rGO and mesoporous carbon by 100 and 60 times, respectively. Building on this foundation, a myriad of carbon-based SAzymes, replicating the active sites found in natural enzymes, have exhibited remarkable enzyme-like activities [39]. In 2019, Dong et al. shed light on the catalytic behavior of SAzyme harboring an FeN₅ active center, strategically confined within a carbon nanoframework [40]. This architecture mirrors the axial ligand coordination reminiscent of heme in cytochrome P450. The introduction of an additional axial ligand N imparts heightened electron-driving effects to Fe–N₅ in comparison to Fe–N₄, resulting in superior oxidase (OXD)-like activity. In 2020, Yan et al. delved into the intricacies of auxiliary factors in natural enzymes, culminating in the design of a POD-like nanozyme comprised of Fe–N₄ and Fe atom clusters [41]. This nanozyme exhibited a spectrum of enzyme-like activities, encompassing superoxide dismutase (SOD), catalase (CAT), urea oxidase (UOD), POD, and OXD-like activity. Fast-forwarding to 2021, Chen et al. synthesized a transition metal nitride, TiN, showcasing remarkable POD-like activity [42]. TiN also demonstrated robust light absorption in both the near-infrared (NIR) I and II regions. Under NIR light irradiation, the POD-like activity of TiN experienced further augmentation due to temperature effects and surface plasmon resonance. To harness these properties, a pH-responsive polyethylene glycol-modified glucose oxidase was covalently linked to lipid vesicles encapsulating TiN nanozymes. This innovative construction resulted in a pH-responsive cascading catalytic nanocomposite that effectively inhibited tumors through synergistic catalytic activities.

Furthermore, N regulated nanozymes have garnered escalating

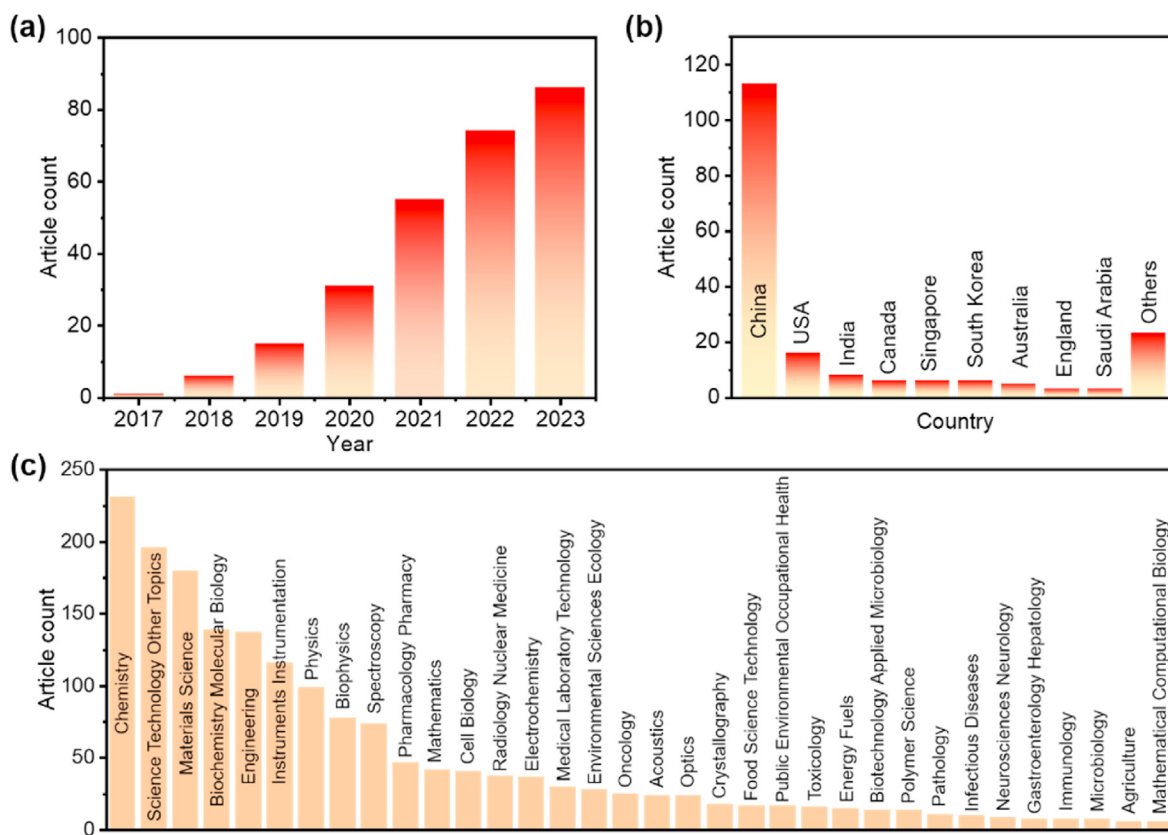


Fig. 1. (a–b) Statistics on the amount of publications related to the topics of nanozymes and nitrogen from 2017 to 2023 (a) and from different country (b). (c) Statistics of publications related to nanozyme and nitrogen in different fields. The data were collected on Web of Science with the keywords of “nanozyme” and “nitrogen”.

interest within the scientific community, as evidenced by a current corpus surpassing 270 publications, showcasing a consistent year-over-year upward trajectory (Fig. 1a). Predominantly, the research landscape in this domain is notably expansive in China, boasting an impressive publication count exceeding 100, followed closely by the United States and India (Fig. 1b). Additionally, N modulated nanozymes have permeated diverse scientific disciplines, demonstrating their versatility and applicability across a range of fields (Fig. 1c). Against this backdrop, we have undertaken a comprehensive examination of the N element regulated nanozymes, delving into their mechanisms, design principles, and diverse applications, offering an intricate exposition of the latest advances in research. Primarily, we embark on a thorough exploration of the elucidation of N element regulated's impact on the catalytic activity of nanozymes. Through a meticulous analysis of n manifestations in materials, encompassing N vacancies, N doping, N coordination, and nitrides, we explicate their regulatory mechanisms. This detailed examination illuminates how these mechanisms precisely govern the catalytic activity and specificity of nanozymes. Subsequently, we present an exhaustive overview of the prevailing applications of N element regulated nanozymes, spanning realms such as sensing and detection, infection treatment, cancer therapy, and pollutant degradation. Our focus extends to detailing how these nanozymes, guided by N elements, contribute to advancements in diverse fields. In conclusion, we synthesize and prospect the landscape of N element regulated nanozymes, highlighting the challenges they confront in understanding catalytic mechanisms, fine-tuning nanozyme catalytic specificity, expanding the

catalytic scope, harnessing synergistic catalytic effects, conducting toxicity studies, and broadening application domains. This review serves as a comprehensive compendium of current research findings, succinctly summarizing the latest technological strides in the N element driven regulation of nanozymes. Additionally, it outlines future trajectories, envisioning the prospect of achieving precise control over nanozyme performance. The anticipation for the N element driven regulation of nanozymes not only enhances our comprehension of these nanomaterials but also instills optimism for their continual advancement and widespread integration in biosensing, diagnostic and therapeutic applications, and environmental solutions (see Fig. 2).

2. The mechanism of nitrogen element regulation to enhance nanozyme activity

In the first instance, the incorporation of N elements has the potential to instigate the formation of novel active sites within the nanozyme structure or modulate the architecture of catalytic active centers. This process, in turn, orchestrates the regulation of catalytic sites involved in catalytic reactions. The consequential structural modulation assumes a pivotal role in amplifying the stability and reactivity of catalytic active centers, thus contributing to an overarching enhancement of catalytic performance. Secondly, the doping of N elements holds promise for finely adjusting the electronic structure of nanozymes, exerting an influence on the charge distribution within catalytic centers. This adjustment positively impacts the optimization of electron transfer processes during

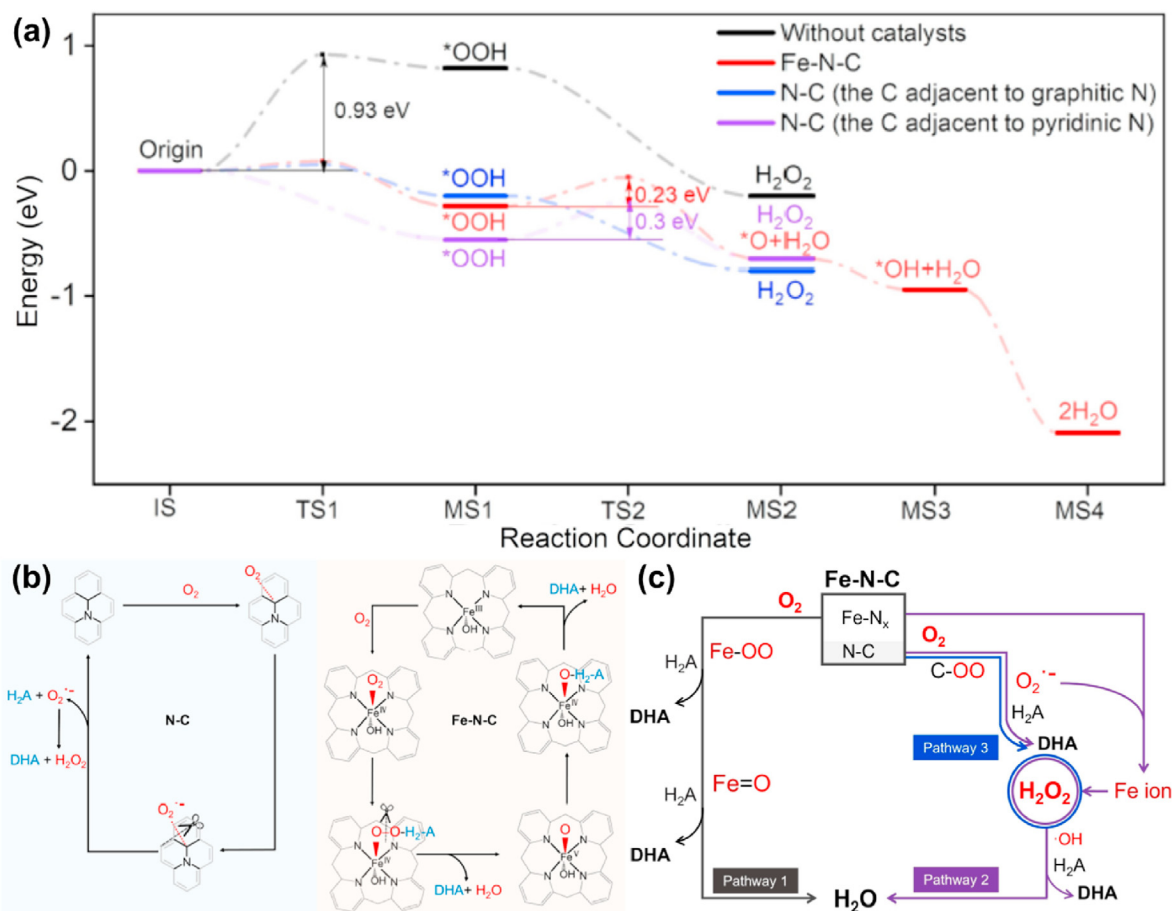


Fig. 2. (a) Energy profiles corresponding to the H₂A oxidation mechanism catalyzed at the Fe-N_x and N-C sites or without catalysis. (b) Scheme of basic ORR pathways catalyzed at the Fe-N_x and N-C sites. (c) Scheme of catalytic H₂A oxidation and oxygen reduction by Fe-N-C. Pathway 1: Boun iron-oxo intermediate pathway at the Fe-N_x sites. Pathway 2: Free ROS intermediate pathway at the N-C sites along with leaching of Fe ions. Pathway 3: Free ROS intermediate pathway producing H₂O₂ at N-C sites without Fe ions, which was proposed as the origin of the pro-oxidant effect of H₂A. (Reproduced with permission from Ref. [16]. Copyright 2023, Wiley-VCH).

reactions, resulting in an augmentation of catalytic activity. Particularly noteworthy is the capability of N elements to fine-tune the band structure of nanozymes, facilitating electron transfer to propel catalytic reactions. Moreover, the regulation of N elements may instigate alterations in the interactions between nanozymes and substrates, augmenting the adsorption capacity of substrates in close proximity to the catalytic center. This augmentation serves to elevate the frequency of contact between substrate molecules and the catalytic center, thereby expediting the progression of catalytic reactions [43]. Additionally, the modulation of N elements has the potential to modify the surface chemical properties of nanozymes, encompassing characteristics such as hydrophilicity and hydrophobicity. These alterations impact the adsorption of substrate molecules on the surface and consequently influence the advancement of catalytic reactions.

The introduction of N elements also contributes positively to enhancing the selectivity of nanozymes [44]. Zhang et al.'s study demonstrates that Fe–N–C nanozyme acts as a multiphase catalyst with both N–C and Fe–N_x active sites [16]. Within Fe–N–C, the Fe–N_x sites catalyze the oxidation of ascorbic acid and the four-electron oxygen reduction via intermediate states, while a small fraction of N–C sites catalyze the two-electron oxygen reduction. During catalysis, trace amounts of superoxide anions are generated, inducing the release of unstable Fe–N_x species in Fe–N–C into Fe ions. Subsequently, the hydrogen peroxide produced by N–C site catalysis reacts with free Fe ions to form a Fenton-like system, triggering a new four-electron oxygen reduction process. Most importantly, when free Fe ions move away from the catalyst, the decrease in Fe–N_x active site content during the ascorbic acid catalytic oxidation process activates more N–C active sites, directly leading to an increase in hydrogen peroxide production. This study unveils, for the first time, the phenomenon of Fe ion precipitation during the ascorbic acid oxidation process catalyzed by Fe–N–C nanozymes, and reveals a novel pathway for oxygen reduction, offering new insights into understanding the application of ascorbic acid-induced oxidation for clearing cancer cells.

3. Design of nanozymes regulated by nitrogen element

The modulation of nanozymes by N elements takes diverse forms, encompassing N vacancies, N doping, N coordination, and nitrides. The implementation of these methodologies typically involves techniques such as etching, solvent thermal treatment, template-assisted synthesis, and calcination. Etching serves as a post-synthesis treatment strategy aimed at introducing defects into nano-catalysts, often employed for nitrogen vacancy introduction. For instance, Zhan et al. successfully prepared N-doped carbon quantum dot nanozymes with high nitrogen vacancies using propylene glycol-assisted ultrasonic fragmentation [45]. N doping is commonly achieved through solvent thermal treatment and calcination techniques [46]. Yan et al. employed the calcination method and utilized F127 soft templates containing melamine, formaldehyde/phenol as nitrogen and carbon sources, respectively, to successfully synthesize nitrogen-doped carbon porous nanospheres (N-PCNs) with a diameter of approximately 100 ± 10 nm [40]. Nanozymes with N coordination are typically realized through solvent thermal and pyrolysis techniques, where solvent thermal treatment is employed to form metal-organic framework materials, followed by pyrolysis to achieve nitrogen-metal coordination. A certain research team first prepared ZIF-8 using solvent thermal treatment and then utilized P as a precursor in the synthesis system to anchor metal active centers and adjust the local coordination structure [47]. Subsequently, Fe/ZIF-8@PZM core-shell composite materials were prepared by polymerizing Fe ions and poly (tris (4,4'-diaminodiphenyl ether) phosphazene) (PZM) monomers on the surface of ZIF-8. Under flowing nitrogen atmosphere, Fe/ZIF@PZM was pyrolyzed at 950 °C for 3 h to obtain FeN₃P-SAzyme. Nitrides are typically achieved through calcination, often using metal oxides or sulfides calcined in ammonia gas. Chen et al. successfully prepared TiN nanozymes by calcining TiO₂ nanoparticles in

an ammonia atmosphere, and further precipitation yielded titanium nitride nanoparticles with strong enzymatic activity [42].

3.1. Nitrogen vacancy

The innovative manipulation of nanozyme activity through N vacancies represents a pioneering approach, enabling precise control over the catalytic performance of nanozymes by incorporating N vacancies into the catalyst structure. The crux of this strategy involves the intentional modulation of the electronic structure and chemical milieu of catalytic active sites through the introduction of N vacancies either on the catalyst's surface or within its structure. This regulatory mechanism not only amplifies the catalyst's adsorption capabilities, resulting in heightened reaction rates but also facilitates the fine-tuning of reaction pathways, providing the means for exacting control over product selectivity.

Graphitic carbon nitride (g-C₃N₄) emerges as a non-metallic polymer akin to graphite, showcasing a π -conjugated network architecture formed by triazine rings. Renowned for its remarkable chemical and thermal stability, distinctive electronic configuration, and eco-friendly attributes, g-C₃N₄ has captivated attention as a benign natural enzyme mimic. While pure g-C₃N₄ lacks inherent enzymatic prowess, the introduction of defects provides a means to finely adjust its electronic structure, thereby enabling the manifestation of nanozyme activity. In the study conducted by Zhan et al., a straightforward ultrasound-assisted approach utilizing propylene glycol was deployed to successfully fabricate nanozymes in the form of g-C₃N₄ quantum dots (CNQDs) with uniform dimensions ranging from 4 to 5 nm, featuring N vacancies (Fig. 3a–b) [45]. The modulation of processing time allowed precise control over the N vacancy content in the synthesized CNQDs (Fig. 3c). Density functional theory (DFT) calculations unveiled a substantial reduction in the intrinsic bandgap of CNQDs (Fig. 3d). Charge analysis disclosed that the carbon valence electrons in the defect-free structure measured 2.366 eV and 2.506 eV. Following the introduction of N vacancies, these values increased to 2.832 eV and 2.847 eV (Fig. 3e). Subsequent DFT calculations were employed to delve into the H₂O₂ activation process. Structural models detailing the absorption of H₂O₂ on CN and CNQDs were established through DFT calculations. The computed results indicated H₂O₂ adsorption energies of –2.265 eV and –7.432 eV for CN and CNQDs, respectively (Fig. 3f). These outcomes underscored that CNQDs, featuring N vacancies, displayed remarkable POD-like catalytic efficiency.

3.2. Nitrogen doping

The modulation of nanozyme activity via N doping stands as a captivating avenue of research. Incorporating N elements into the catalyst structure introduces novel active sites. Furthermore, by manipulating the surface electronic structure, chemical affinity, and surface acidity/basicity of the catalyst, diverse opportunities emerge for augmenting the activity and selectivity of the catalyst [48].

Carbon materials, renowned for their exceptional biocompatibility, have witnessed widespread applications in the realm of biomedicine. Typically, carbon-based nanozymes undergo nitrogen doping, wherein N atoms replace original carbon atoms, thereby aiming to enhance or elevate their enzymatic activity. In a study by Yan et al., N-doped carbon porous nanospheres (N-PCNs) were synthesized with a diameter of approximately 100 ± 10 nm using a calcination method [49]. The fabrication involved a soft template of F127 containing melamine, along with formaldehyde/phenol as N and C sources (Fig. 4a). The repercussions of N doping were systematically explored (Fig. 4b), revealing that N-PCNs demonstrated four distinct enzyme activities: OXD, POD, CAT, and SOD (Fig. 4c). Upon the introduction of ferritin, N-PCNs effectively targeted TfR1-positive tumors by specifically binding with ferritin and its receptor, leading to their localization in lysosomes. Within the lysosomal environment, the varied enzyme activities of N-PCNs facilitated the consumption of O₂ and H₂O₂, resulting in the generation of

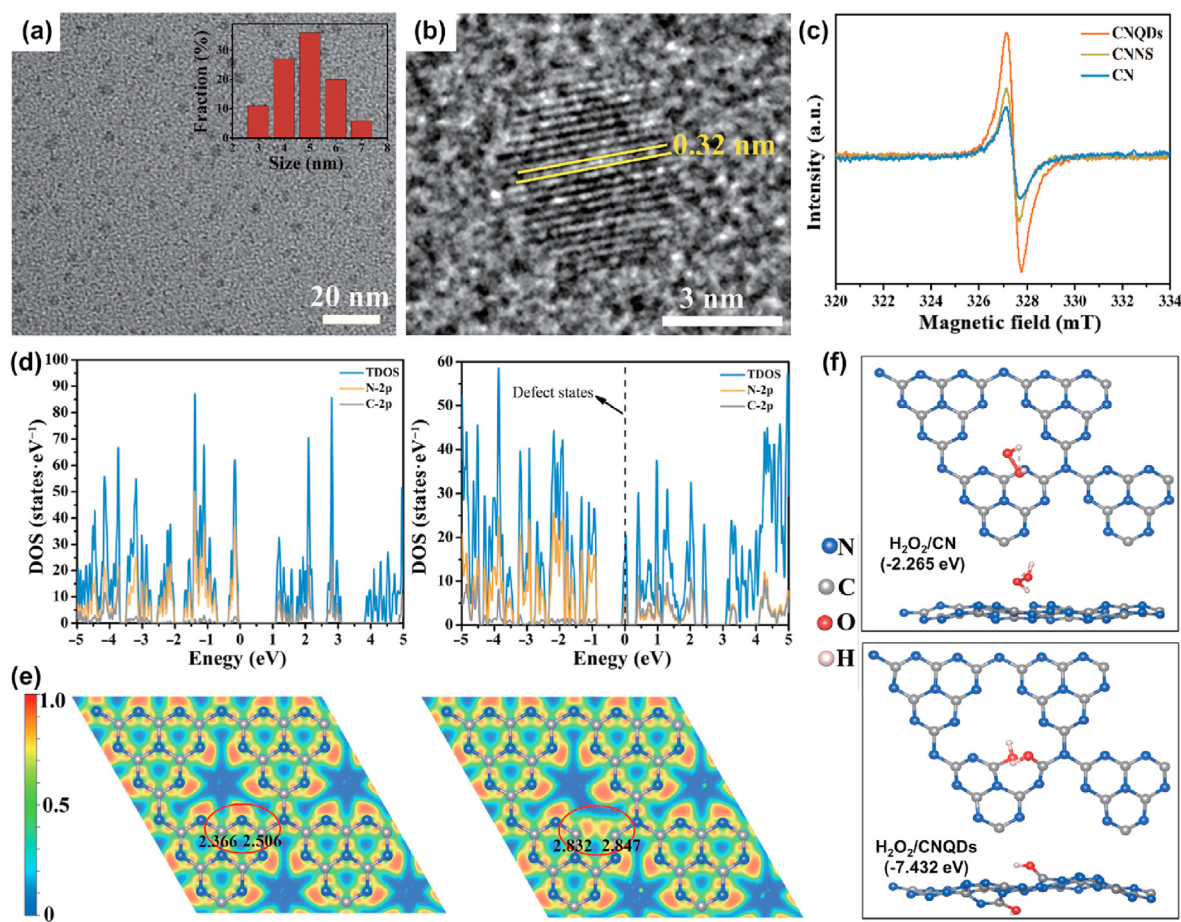


Fig. 3. (a–b) TEM (a) and HRTEM (b) images of CNQDs. (c) EPR spectra of the CN, CNNS, and CNQDs. (d–f) The density of state (DOS) (d), ELF (e) and the structure models and adsorption energy values (f) for absorbing H₂O₂ based on DFT calculation of CN and CNQDs. (Reproduced with permission from Ref. [45]. Copyright 2023, Springer Nature).

increased reactive oxygen species (ROS). This collaborative modulation of ROS levels induced cellular damage, ultimately culminating in tumor regression. This study underscores the capacity of N-PCNs to regulate intracellular reactive oxygen species through the emulation of diverse enzyme-like activities, harnessing Fe ammonia for targeted tumor therapy *in vivo* and positioning them as a promising nanocatalyst.

Nevertheless, the instability of N at elevated calcination temperatures poses a persistent challenge in the quest for carbon nanozymes with substantial N content. Wei et al. have introduced an innovative N-doping strategy, employing a N-rich polymer, polyethyleneimine (PEI), as the N source and utilizing the natural clay mineral montmorillonite (MMT) as a template to fabricate highly active POD-like carbon nanozymes (Fig. 4d) [50]. The MMT-PEI assembly serves as a protective barrier against the loss of N elements during high-temperature calcination, thus preserving a greater number of catalytically active N sites. Hydroxyl radicals, pivotal intermediates in POD-like catalysis, were examined using a highly active and specific carbon nanozyme for the detection of H₂O₂, glucose, and ascorbic acid (Fig. 4e). Additionally, the total antioxidant capacity of four commercial beverages was evaluated. This research not only introduces a novel strategy for the preparation of POD-like nanozymes but also presents a convenient method for determining total antioxidant capacity, with potential applications in assessing the quality and oxidative stress of antioxidant-rich foods in the realm of healthcare. In a parallel study, Wang et al. employed a plasma-assisted strategy to incorporate N heteroatoms (N) into quaternized graphene (QG) at ambient temperature, yielding a carbon-based green nanozyme (Fig. 4f) [51]. The investigation revealed that the resulting N-doped QG (N-QG) nanozyme displayed significantly heightened catalytic activity, nearly five times greater than

that of the original QG, as substantiated by kinetic studies (Fig. 4g). In this context, plasma-assisted N-doping enhanced the conductivity (hydrophilicity) of QG, introduced surface defects, facilitated electron transfer, and amplified the catalytic activity of N-QG. Furthermore, the exploration of POD, SOD, and OXD-like catalytic activities of N-QG indicated that N doping conferred the obtained nanozyme with highly specific POD-like catalytic activity. The subsequent colorimetric analysis of H₂O₂ in milk samples validated the applicability of the developed N-QG nanozyme. Importantly, this plasma-assisted heteroatom doping approach holds the promise to revolutionize the rational design of diverse enzyme-mimicking entities, enhancing catalytic performance on a large scale.

3.3. Nitrogen coordination

The catalytic activity of nanozymes can be intricately modulated through the exact coordination of metals with N elements [52]. This regulatory coordination not only assists in refining the structure of catalytic active centers, augmenting their substrate adsorption capabilities, but also facilitates the fine-tuning of the electronic structure, thereby impacting catalytic performance [53]. This distinctive metal-N coordination strategy not only furnishes meticulous and microscopic control over the catalytic activity of nanozymes but also serves as a potent instrument for the focused design and optimization of nanozyme performance [54,55].

The nitrogen coordination number and type are pivotal factors influencing nanozyme activity. Presently, the prevalent approach involves fabricating single-atom nanozymes, where the enzymatic activity

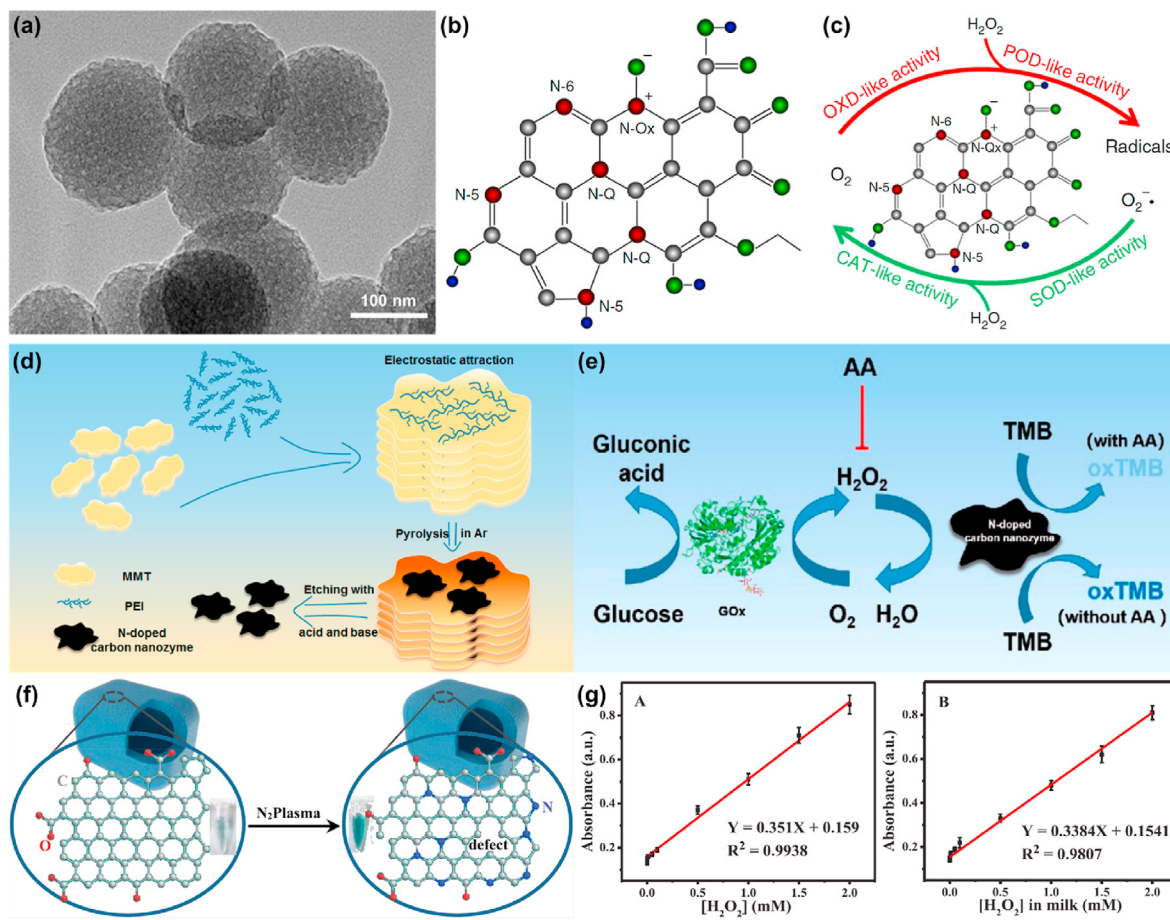


Fig. 4. (a) HRTEM image of N-PCNSs-3. (b) Schematic model of N and O containing surface functionality in N-PCNSs-3. (c) Schematic presentation of enzyme-like activities of N-PCNSs. (Reproduced with permission from Ref. [49]. Copyright 2018, Springer Nature). (d) Schematic illustration of the synthesis process of N-doped carbon sheets based on the strong electrostatic attraction between MMT and PEI. (e) Schematic illustration of detecting H_2O_2 , glucose and AA by using a POD-like N-doped carbon nanozyme (PDB code of GOx: 1CF3). (Reproduced with permission from Ref. [50]. Copyright 2019, American Chemical Society). (f) Schematic illustration of the preparation of NQ by the N_2 plasma treatment for QG. (g) Calibration curves of the relationship between the absorbance responses and different concentrations of H_2O_2 in (A) buffer and (B) milk samples. (Reproduced with permission from Ref. [51]. Copyright 2020, American Chemical Society).

is modulated by adjusting the coordination between metal atoms and nitrogen. However, due to variations in preparation methods and the types/densities of metal-nitrogen atoms, there is yet no standardized criterion to definitively determine which coordination is most conducive to nanozyme activity. Below, we elucidate how the nitrogen coordination number and type affect nanozyme activity through specific examples. For instance, Zhang et al. thoroughly investigated the general catalytic properties of Fe–N–C artificial enzymes under mild conditions, demonstrating their near 100% selectivity in directly utilizing O_2 to oxidize substrates. Their research revealed that the catalytic activity of Fe–N–C could be effectively modulated by adjusting the type of precursor for pyrolysis [56]. Compared to traditional heterogeneous and homogeneous artificial enzymes, Fe–N–C exhibits several notable advantages, including durability in extreme pH and high temperatures, ease of separation and recycling, and the ability to utilize O_2 directly without the need for sacrificial oxygen donors such as H_2O_2 . These findings open up new possibilities for the application of Fe–N–C across various fields.

Li et al. engineered a FeN_3P SAzyme with POD-like activity [47]. Through precise coordination of N and P elements, they finely tuned the electronic structure of the single-atom Fe active center, creating an active center reminiscent of natural horseradish POD (Fig. 5a). Moreover, by quantitatively measuring the absorbance intensity of the colorimetric reaction catalyzed by the nanozyme, specific activity values were determined. FeN_3P SAzyme demonstrated superior catalytic activity compared to the widely utilized Fe_3O_4 nanozyme or FeN_4 SAzyme

lacking P coordination (Fig. 5b). Furthermore, the mechanism behind the elevated POD-like activity of FeN_3P SAzyme was probed using DFT calculations. As depicted in Fig. 5c, the energy distribution of the optimal pathway for the oxidation of POD substrates by surface O species generated from H_2O_2 decomposition was computed. In this scenario, FeN_3P SAzyme displayed lower barriers for the formation of surface O and OH species (<0.5 eV), whereas FeN_4 SAzyme and $\text{Fe}_3\text{O}_4(111)$ exhibited almost negligible POD-like activity under acidic conditions. For FeN_4 SAzyme, the surface OH species, being challenging to transform into a H_2O molecule (1.05 eV), remained attached to the active center of the Fe atom, thereby impeding the entire catalytic process.

However, the synthesis of the majority of SAzymes still encounters challenges, including issues of aggregation and the precise optimization of local coordination structures. The spatial confinement strategy seeks to overcome these challenges by inhibiting the aggregation and migration of atoms during the pyrolysis process, thereby achieving well-dispersed SAzymes. Huang et al. pioneered the development of a Fe SAzymes with N coordination confined within a carbon nanoframe (Fe– N_5 SA/CNF) [40]. Through the pyrolysis of MOF-encapsulated Fe phthalocyanine at 900°C under a N_2 atmosphere, they successfully constrained isolated FeN_4 sites within the carbon nanoframe, yielding thermodynamically more stable FeN_5/C sites that emulate the active site of natural cytochrome P450 (Fig. 5d). Moreover, the oxidase-like activity of FeN_5 SA/CNF was evaluated using a colorimetric assay, employing the oxidation of TMB as a model catalytic reaction to investigate the

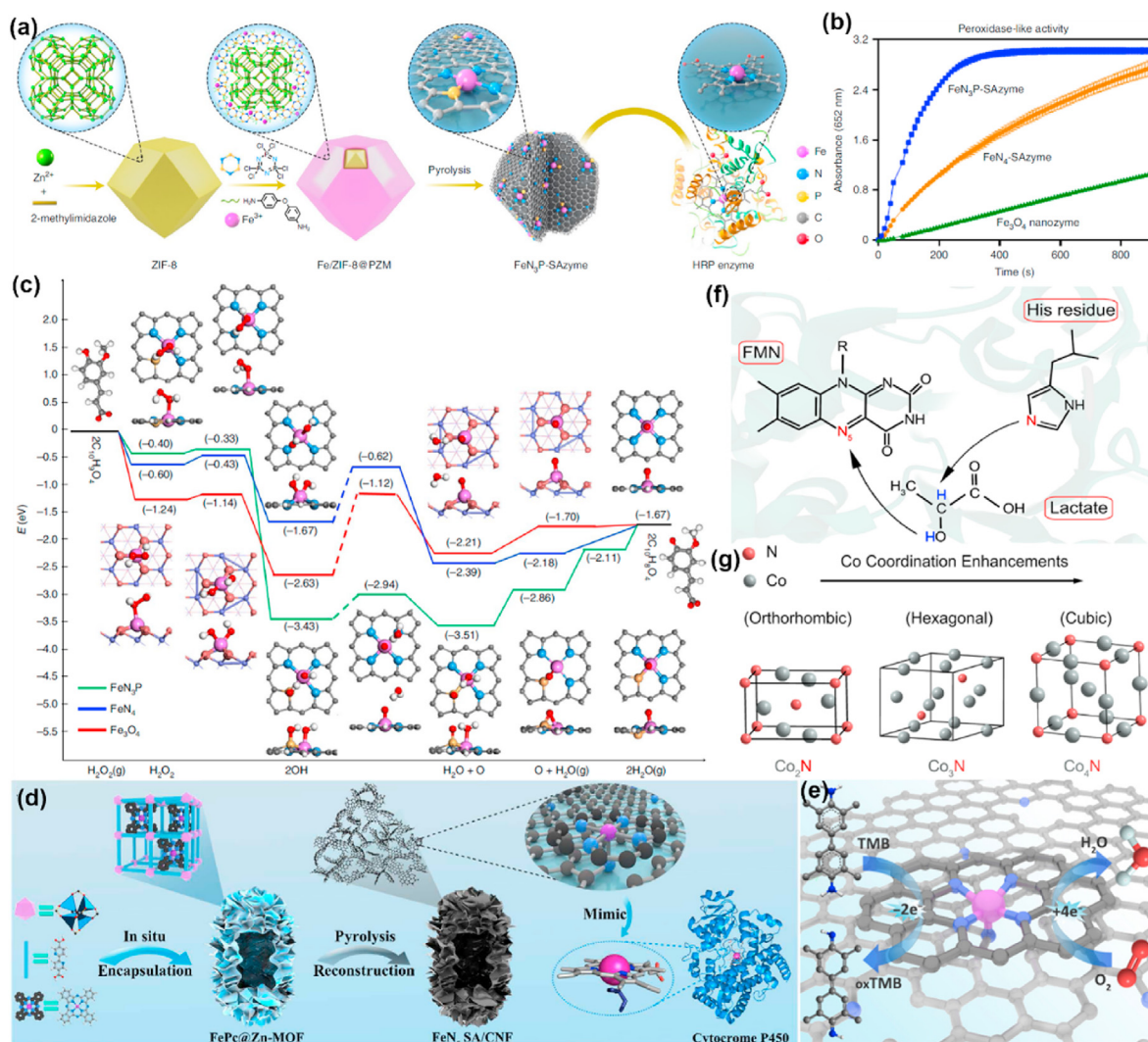


Fig. 5. (a) Illustration of the preparation process of FeN₃P SAzyme. (b) Reaction-time curves of the TMB colorimetric reaction catalyzed by FeN₃P SAzyme, FeN₄ SAzyme, and Fe₃O₄ nanozyme. Error bars represent s.d. obtained from three independent experiments. (c) DFT studies on the POD-like activity of FeN₃P-SAzyme, FeN₄ SAzyme, and Fe₃O₄ nanozyme. The energy profile diagram shows the most favourable paths of H₂O₂ dissociation into surface O species in neutral conditions, as well as the reaction 2C₁₀H₉O₄+O → 2C₁₀H₈O₄+H₂O(g), in which the transition states are not included. Colour code: Fe, blue; C, grey; N, light blue; P, yellow; O, light red; H, white. To make a distinction between the active sites of the Fe and O atoms, they are shown in pink and red, respectively. (Reproduced with permission from Ref. [47]. Copyright 2021, Springer Nature). (d) Schematic formation process of carbon nanoframe-confined atomically dispersed Fe sites with axial 5-N coordination for mimicking the active center of cytochrome P450. (e) Schematic illustration of oxidase-like characteristics of FeN₃ SA/CNF-catalyzed TMB oxidation. Reproduced with permission from Ref. [40]. Copyright 2021, AAAS. (f) Proton abstraction from lactate toward natural LOX based on carbanion formation mechanism (PDB code: 5EBU). (g) Crystal structures of Co₂N, Co₃N, and Co₄N. (Reproduced with permission from Ref. [57]. Copyright 2023, American Chemical Society).

interaction between O₂ molecules and FeN₅ SA/CNF in various environments. The results demonstrate the exceptional POD-like catalytic activity of this SAzyme. In a separate study, Liu et al. designed and synthesized a five-coordinated FeN₅ SAzyme with an activity structure akin to natural enzymes [58]. The surface of zeolitic imidazolate framework precursors was coated with an SiO₂ shell, preventing material aggregation during high-temperature pyrolysis and imparting the resulting carbon nanoparticles with a superior porous structure and higher surface area. Notably, the catalytic efficiency constant (k_{cat}/K_m) of the FeN₅ SAzymes is 7.64 times higher and 3.45×10^5 times higher than that of traditional FeN₅ SAzymes and commercial Fe₃O₄ nanozymes, respectively.

The catalytic activity of nanozymes is commonly regulated by manipulating the metal center and non-metal ligands within the nanozyme structure. However, this paradigm does not seamlessly apply to lactate oxidase, a flavoenzyme with a flavin mononucleotide (FMN)-dependent pathway. Liu et al. introduced a coordination strategy to

mimic lactate oxidase, involving the adjustment of the number of Co atoms proximal to N in the Co_x-N nano-composite material to modulate the electronic characteristics of the N center (Fig. 5f–g) [57]. Capitalizing on the coordinated field and electronic structure surrounding the electron-rich N sites, Co₄N/C precisely identifies lactate and intermediate tissue sites, optimizing the absorption energy of intermediates and facilitating the oxidation of lactate α-C-sp³-H bonds into ketones. This optimized nanozyme, by reversing the elevated lactate and immune-suppressive conditions of the tumor microenvironment, dramatically enhances the anticancer effect, resulting in remarkable inhibition of tumor growth and distant metastasis. The development of Co₄N/C nanoparticles introduces novel prospects that bridge the gap between chemical and biological catalysis. This nanozyme design strategy not only contributes to comprehending the evolution of nanozymes but also inspires the construction of the next generation of artificial enzymes. Lu et al. uncovered that the POD-like activity of Co SAzymes is substantially influenced by the local N coordination environment

surrounding the Co single-atom catalytic site [59]. The coordination number of N atoms can effectively modulate the O₂ adsorption performance of Co atoms, thereby impacting their POD-like activity. Furthermore, a series of Co SAzymes with varying N coordination numbers (Co-N_x(C), $x = 2, 3, 4$) exhibited a clear relationship between N coordination and POD-like activity, as revealed through DFT calculations and enzyme activity analyses. Among the investigated single-atom Co catalysts, Co-N₃(C) with three coordinated N atoms displayed the optimal oxygen adsorption structure and robust ROS generation capacity, showcasing superior POD-like activity.

3.4. Nitride

Additionally, drawing inspiration from the synergistic coordination observed in the catalytic active sites of biological enzymes involving metal and N, Chen et al. achieved successful synthesis of transition metal nitrides [42]. They achieved the nitridation of TiO₂ nanoparticles under an ammonia atmosphere, yielding TiN (Fig. 6a). DFT simulations were employed to calculate the adsorption energies of various intermediates during the H₂O₂ decomposition process (Fig. 6b). Notably, the H₂O₂ decomposition on TiN exhibited consistently negative adsorption energies, indicative of exceptional POD-like enzyme activity. This

phenomenon is attributed to the lower electronegativity of N atoms compared to oxygen atoms, creating an optimal environment for titanium to manifest enhanced POD-like activity (Fig. 6c). As the extent of nitridation increases, the nanozyme activity of TiN proportionally improves. Upon complete nitridation of TiO₂ into TiN, it demonstrates the highest POD-like activity (Fig. 6d). Moreover, TiN displays pronounced light absorption in both the NIR I and II regions. Leveraging temperature effects and surface plasmon resonance, TiN nanozyme activity is further augmented under NIR light irradiation (Fig. 6e).

However, certain nitrides, exemplified by the previously discussed pristine g-C₃N₄, inherently lack autonomous catalytic activity. Nevertheless, their catalytic potential can be augmented by the introduction of N vacancies. Beyond the incorporation of N vacancies, surface modification emerges as a viable strategy to bestow catalytic activity upon g-C₃N₄. Choi et al. employed a one-pot thermal condensation approach to synthesize unaltered g-C₃N₄, followed by subsequent surface modification using KOH and KCl to yield surface-modified g-C₃N₄ (AKCN) (Fig. 6f) [60]. On the surface of AKCN, hydroxyl groups (–C–OH) were identified, signifying the replacement of terminal –NH₂ groups with hydroxyl groups subsequent to the introduction of KCl and KOH. Additionally, AKCN effectively integrated K and Cl atoms into the C₃N₄ framework. DFT calculations elucidate that the introduction of OH

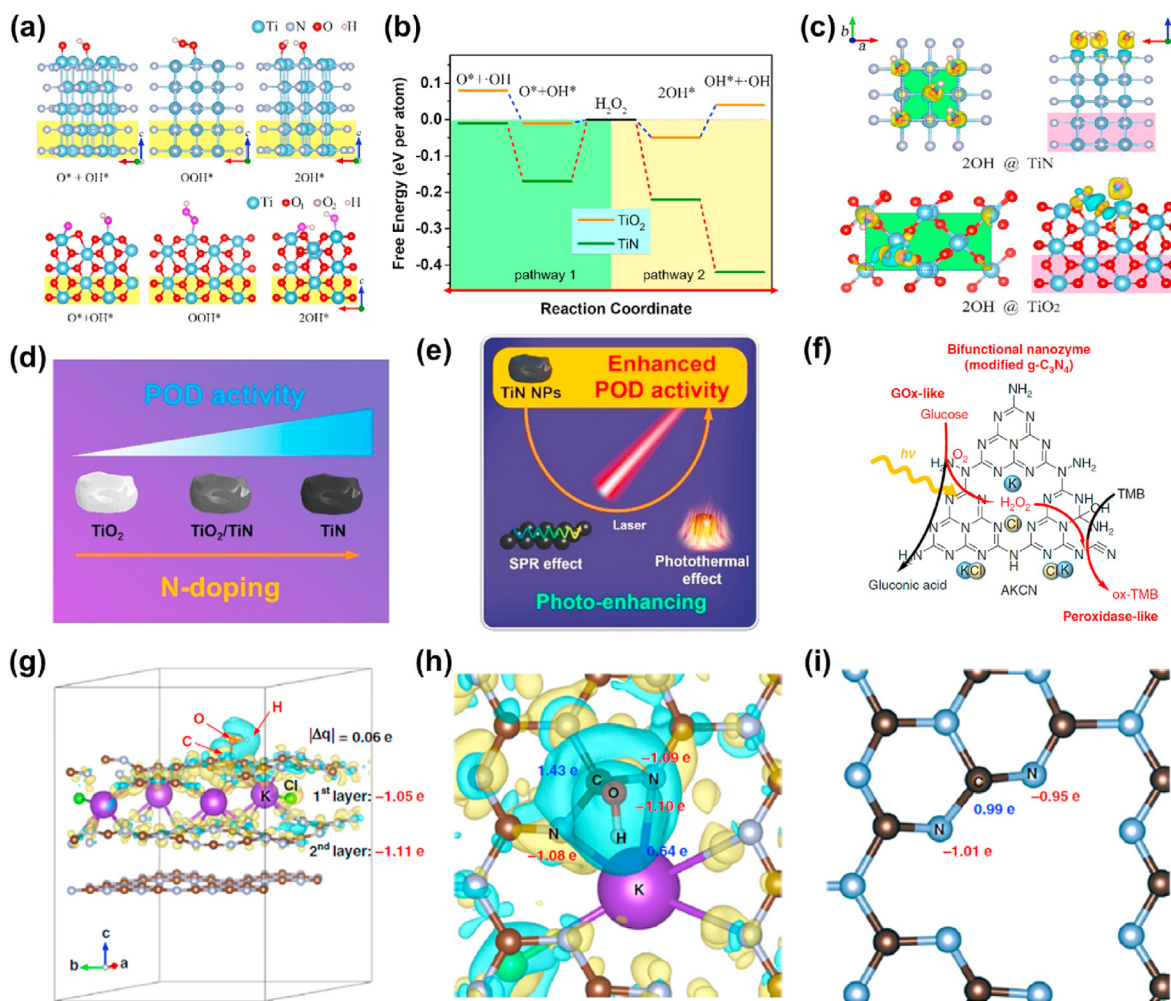


Fig. 6. (a) Schematic of TiN and TiO₂ models. (b) The free energy diagrams of TiN and TiO₂ to produce catalyst hydroxyl radicals. (c) Different charge density of TiN (001) and TiO₂ (001) surfaces. Blue and yellow represent charge loss and charge accumulation, respectively. The isosurface value is set to 0.01 Å⁻³. (d) Effect of N-doping on the POD activity of Ti-based nanozymes and mechanism investigation. (e) Schematic illustration to show the photo-enhanced POD activity of TiN NPs. (Reproduced with permission from Ref. [42]. Copyright 2021, Wiley-VCH). (f) Glucose detection using a synthetic bifunctional nanozyme. (g–i) Charge distribution of KCl–OH–GCN (g), an enlarged top view of KCl–OH–GCN (h), and that of pristine g-C₃N₄ (i). (Reproduced with permission from Ref. [60]. Copyright 2019, Springer Nature).

groups into AKCN prompts hydroxyl groups to preferentially bond with surface carbon, resulting in the formation of a notable electron-depleted region around the hydroxyl groups in the first layer (Fig. 6g). This, in turn, diminishes $|\Delta q|$ from 0.16 e to 0.06 e, thereby facilitating a more relaxed charge transfer between adjacent layers. Furthermore, DFT calculations disclose that the presence of OH groups induces alterations in the charge distribution across different atoms. The magnified top view of KCl-OH-GCN (Fig. 6h) demonstrates that the surface carbon atom connected to the OH group carries a more positive charge compared to the corresponding C atom in pristine GCN. Consequently, neighboring N atoms in KCl-OH-GCN bear more negative charges than those in pristine GCN (Fig. 6i).

4. Practical applications of nitrogen element regulation on nanozymes

4.1. Sensing detection

Nanozymes modulated by N elements present a promising landscape for extensive applications in the fabrication of cutting-edge sensing platforms [61]. This opens up novel avenues for swiftly and precisely detecting biomolecules and conducting environmental monitoring [62, 63]. Advancements in this domain not only enhance our comprehension of the regulatory mechanisms involving N elements but also drive pioneering strides in the utilization of nanozymes for biological and chemical sensing applications [64].

Wang et al. employed N-rGO, and DFT calculations revealed that N-rGO exhibits a more pronounced exothermic process during the desorption of hydroxyl radicals compared to undoped graphene, thereby conferring POD-like activity to the material (Fig. 7a) [65]. Additionally, on the N-rGO surface, molybdenum disulfide nanosheets with sulfur and molybdenum vacancies were grown, enhancing its enzyme activity. Subsequently, a straightforward and sensitive colorimetric method was devised for the quantitative detection of glucose and GSH, achieving detection limits as low as 0.02 μM and 0.12 μM , respectively (Fig. 7b and c). This innovative strategy not only opens a new avenue for the synthesis of efficient nanozymes but also presents a novel approach for the sensitive detection of various biomolecules under physiological conditions in the future. Cystathionine γ -lyase (CSE) exhibits elevated expression levels in diverse cancers, including breast cancer, glioblastoma, and ovarian cancer. Wei et al. introduced a novel technique utilizing Ru-N-C nanozymes for CSE detection. Initially, Ru-N-C nanozymes were engineered with exceptional POD-like activity (Fig. 7d) [66]. Subsequently, a tumor model was established in nude mice by implanting HepG2 cells to validate the potential of the Ru-N-C nanozyme-based CSE biosensing platform in tumor tissues (Fig. 7e). After euthanizing the tumor-bearing mice, tumor tissues were collected for the subsequent Ru-N-C nanozyme-based CSE activity assay. Following the identical experimental protocol used for tumor cell analysis, the tumor tissues underwent lysis and subsequent treatment with cystathionine and PLP. The results illustrated in Fig. 7f underscore the adaptability and versatility of the Ru-N-C nanozyme-based CSE assay for ex vivo analysis of tumor tissues.

Moreover, nanozymes facilitate intracellular detection. Zhu et al. successfully synthesized Fe-N-C SAzymes (Fe-N-C SAzymes) with inherent POD-like activity using FeCl_2 , glucose, and dicyandiamide as precursors [67]. Fe-N-C SAzymes, featuring FeN_x as active sites, resembled natural metalloproteases and selectively enhanced POD-like activity (Fig. 7g). Consequently, owing to the outstanding catalytic efficiency of Fe-N-C SAzymes, colorimetric biosensing of H_2O_2 *in vitro* was executed via a typical TMB-induced chromogenic reaction, demonstrating satisfactory specificity and sensitivity (Fig. 7h and i). In practical applications, *in situ* detection of H_2O_2 generated from HeLa cells by Fe-N-C SAzymes was also performed, broadening the scope of applications for this innovative SAzyme. Li et al. based their investigation on Fe-N co-doped microflowers (Fe-N-C MFs) nanozymes, establishing a straightforward, sensitive, and effective colorimetric sensor for detecting

tannic acid (TA) [68]. Fe-N-C MFs nanozymes with atomically dispersed Fe-N_x active centers were synthesized using a template method and a polyvinylpyrrolidone-assisted synthetic method (Fig. 7j). Under optimized conditions, a colorimetric sensor based on the oxidation enzyme-mimicking activity of Fe-N-C MFs was developed for sensitive TA detection (Fig. 7k). Simultaneously, Fe-N-C MFs nanozymes enabled TA detection through a "thing recognition" application installed on a smartphone.

Furthermore, characterized by coupled catalytic promotion for efficient turnover and excellent specificity, enzyme cascade reactions have generated significant interest in the field of biological analysis. Lu et al. demonstrated that transition metal nitride carbons (f-MNCs, M = Fe, Cu, Mn, Co, Zn) possess intrinsic POD-like activity in formamide conversion, with f-FeNC exhibiting the highest activity, followed by f-CuNC, f-MnNC, f-CoNC, and f-ZnNC [69]. Capitalizing on the exceptional catalytic performance and well-defined catalytic mechanism of f-FeNC, they innovatively devised a multifunctional colorimetric bioanalytical method based on enzyme cascade, catering to the ultra-sensitive detection of glucose and α -glucosidase (α -Glu) associated with diabetes. This multi-enzyme cascade, involving nanozymes without the need for complex enzyme engineering techniques, holds promise for revolutionizing the widespread application of nanozymes in astrophysical detection, disease diagnosis, and biomedicine. Zhang et al. report a copper single-atom catalyst (CuSAC_6N_6) composed of a novel topology of graphitic carbon nitride (C_6N_6) bridged by electron donor- π -acceptor moieties and copper atoms [70]. CuSAC_6N_6 not only achieves basic oxidation reactions via the binding state copper-oxygen intermediate pathway but also triggers a second enhancement reaction under the same conditions through a photoactivated free radical pathway. The unique topology of CuSAC_6N_6 and the electron donor- π -acceptor bridging functional groups facilitate the separation and migration of intramolecular charges, thus eliminating interference from electron transfer between the two catalytic pathways. Experimental results demonstrate that CuSAC_6N_6 not only exhibits outstanding basic activity but also achieves up to 3.6-fold enhancement under indoor lighting conditions. Leveraging the adaptive capability of CuSAC_6N_6 , the team further constructs an *ex vivo* intelligent glucose biosensor with switchable sensitivity and linear detection range. Given its excellent spatiotemporal resolution and high controllability under illumination, the adaptive biosensor reported in this study provides a more integrated dynamic chemical sensing interface for smart artificial devices.

4.2. Infection therapy

In recent times, there has been a growing emphasis on the ramifications of microbial infections for both individual and public health within society [71,72]. Conventional approaches to bacterial infection treatment heavily rely on antibiotics; however, the excessive use of these antibiotics has given rise to antibiotic-resistant "superbugs," presenting a grave peril to public health and the ecosystem [73,74]. Nanozymes, owing to their expansive activity range, outstanding stability, cost-effectiveness, and minimal antimicrobial resistance, are emerging as a highly promising category of novel antimicrobial agents [75].

The research conducted by Yu and collaborators delved deep into the underlying mechanisms of nanozyme activity [76]. To discern whether the POD-like activity emanates from C-S, C-N, or C-N-S coordination, a myriad of models was constructed, including g-CN, g-SCN, graphene, N-graphene, and S-graphene (Fig. 8a-b). Employing DFT calculations, they substantiated that S-N-C coordination serves as the foundational source of POD-like activity (Fig. 8c). Notably, g-SCN not only exhibited remarkable enzymatic catalytic performance but also, courtesy of S doping, mitigated the material's bandgap, broadened the absorption spectrum, and showcased remarkable photocatalytic prowess (Fig. 8d). Capitalizing on the synergistic interplay of photocatalysis and nanozymes, g-SCN demonstrated the ability to catalyze H_2O and low concentrations of H_2O_2 , yielding heightened production of ROS and,

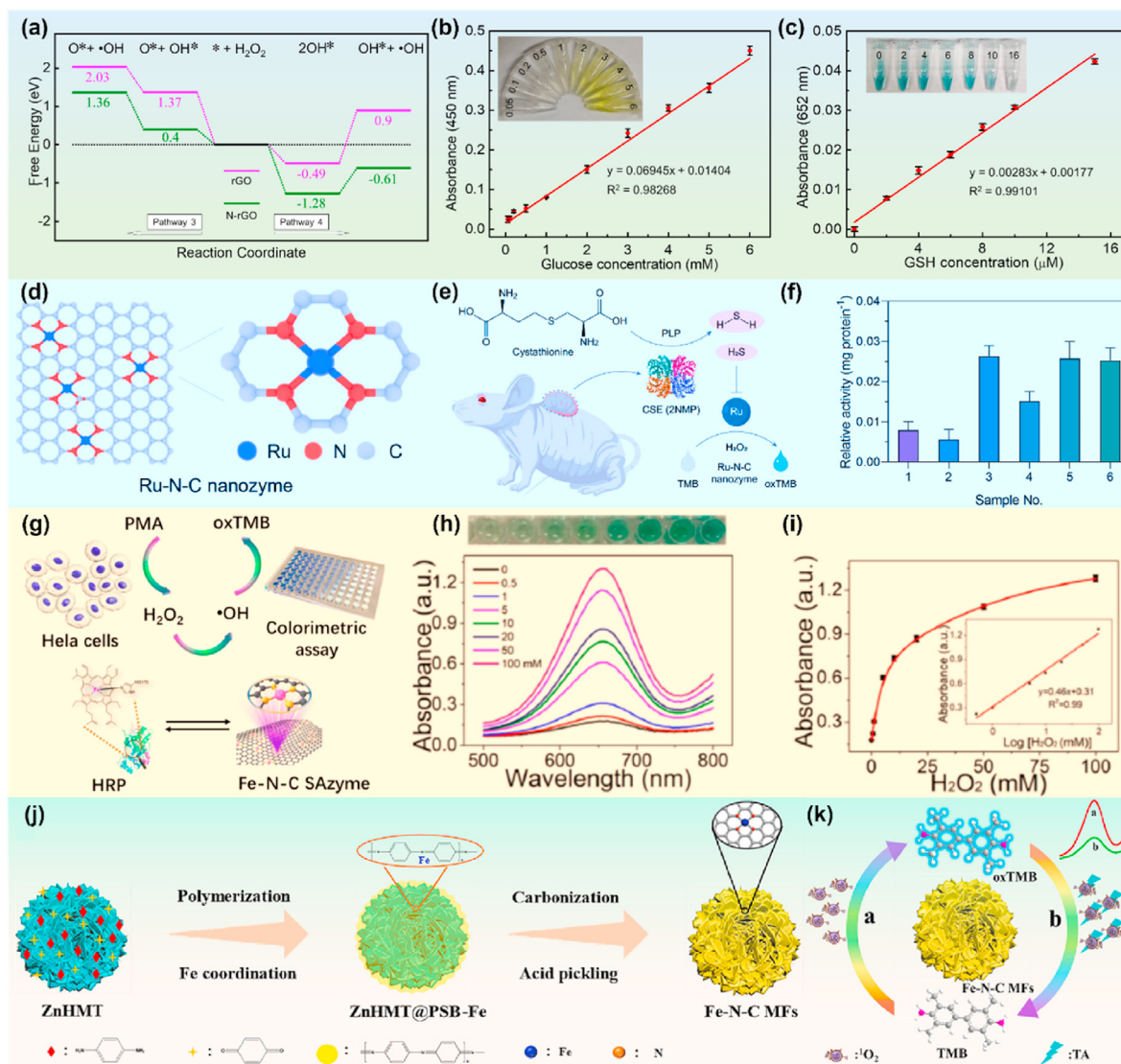


Fig. 7. (a) Free energy diagrams of rGO and N-rGO models for the production of the catalyst hydroxyl radical. Blue and yellow represent charge loss and charge accumulation, respectively, and * denotes the corresponding catalyst (MoS₂ with different models and rGO with and without N doping). (b–c) Glucose (b) and GSH (c) detection based on POD-like MoS₂/N-rGO. (Reproduced with permission from Ref. [65]. Copyright 2021, American Chemical Society). (d) Schematic illustration of the Ru–N–C nanozyme. (e–f) Schematic (e) and performance (f) of the Ru–N–C nanozyme-based biosensing platform for the CSE activity assay in tumor tissues ex vivo. (Reproduced with permission from Ref. [66]. Copyright 2024, Elsevier). (g) Mechanism of H₂O₂ detection released from HeLa cells by Fe–N–C SAzyme. (h) UV–vis absorption spectra of TMB oxidized by the Fe–N–C SAzymes with different concentrations of H₂O₂ and (i) its corresponding calibration curve. (Reproduced with permission from Ref. [67]. Copyright 2019, American Chemical Society). (j) Schematic illustration of the synthesis process of Fe–N–C MFs. (k) Schematic illustration of oxidase mimic characteristics of Fe–N–C MFs for the determination of TA. (Reproduced with permission from Ref. [68]. Copyright 2023, Elsevier).

consequently, outstanding bactericidal properties. The material, showcasing exceptional potential, could potentially serve as a substitute for antibiotics in treating infections in mice, demonstrating remarkable capabilities in wound healing. This study offers substantial backing for the prospective development of innovative treatment modalities.

In a parallel endeavor, Yang and colleagues synthesized a single-atom catalyst with Cu–N–C coordination through a salt template method executed at elevated temperatures [77]. Enriched with active Cu sites, this catalyst exhibited commendable specific oxidase and POD-like activities, thereby significantly amplifying antimicrobial efficacy through the release of O₂^{•−} and $\cdot OH$ (Fig. 8e). The Cu–N–C catalyst demonstrated superior inhibitory effects on various bacteria and displayed the potential to impede the formation of drug-resistant bacterial strains. In a wound model, the prepared Cu–N–C catalyst not only hastened bacterial demise but also facilitated accelerated wound healing (Fig. 8f). This material showcased excellent biocompatibility, underscoring substantial potential

for supplanting traditional antibiotics and antimicrobial materials. Furthermore, Wei and colleagues engineered a spherical mesoporous Fe–N–C SAzyme designed for antimicrobial therapy utilizing photothermal-enhanced Fenton-like catalysis (Fig. 8g) [78]. Capitalizing on a large pore size, high surface area, and uniform diameter, the catalytic performance of Fe–N–C SAzyme experienced significant enhancement. Additionally, owing to its mesoporous structure, Fe–N–C SAzyme demonstrated heightened photothermal conversion efficiency. Under light irradiation, catalytic activity was augmented due to the ensuing rise in reaction temperature. The material also demonstrated the ability to induce bacterial death through physical heat effects. The synergistic amalgamation of nanozyme catalysis and photothermal treatment resulted in antimicrobial performance far surpassing that achievable through a singular antimicrobial approach.

Liu et al. utilizing the ZIF-8 precursor, synthesized an exceptionally active SAzyme featuring a Zn porphyrin structure, employing a

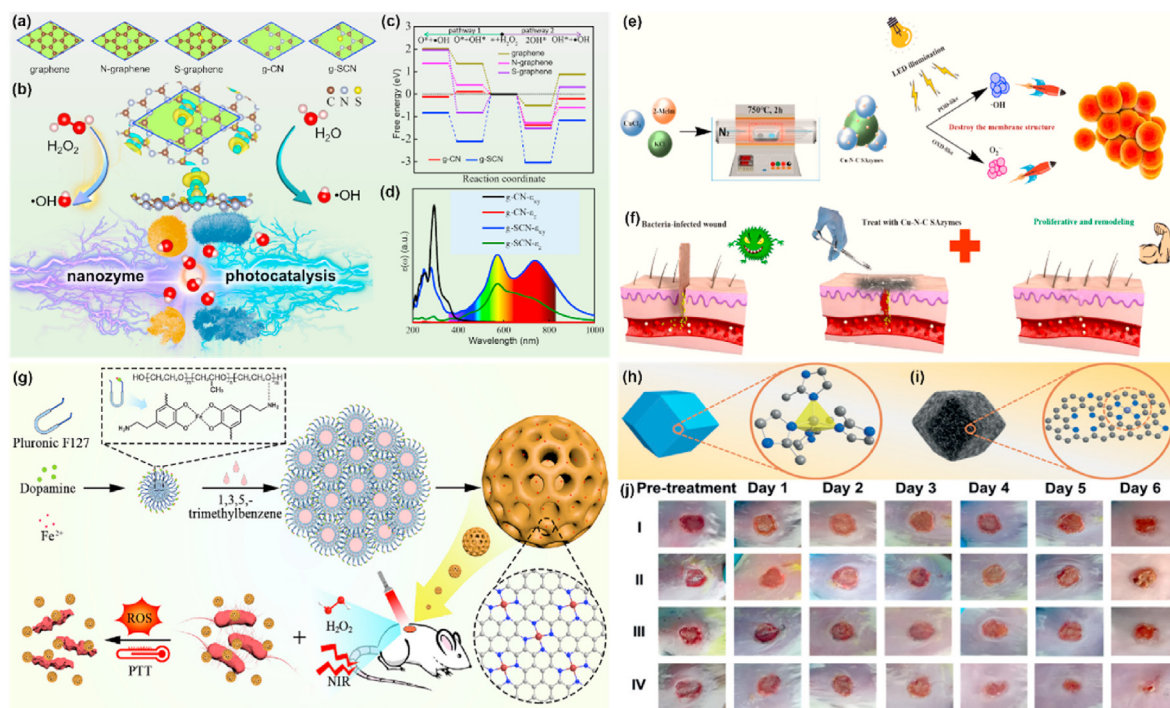


Fig. 8. (a) Schematic illustrations of different models: g-CN, g-SCN, graphene, N-graphene, S-graphene. (b) Schematic diagram of photocatalysis and enzyme synergistic antibacterial therapy. (c) The free energy diagrams of the different models for the production of catalyst hydroxyl radical. (d) Imaginary parts of dielectric constant for g-CN and g-SCN, respectively. (Reproduced with permission from Ref. [76]. Copyright 2023, Elsevier). (e–f) Schematic diagram of the Cu–N–C synthesis process and antibacterial effect (e) and schematic diagram of wound treatment (f). (Reproduced with permission from Ref. [77]. Copyright 2022, American Chemical Society). (g) Schematic illustration of the synthesis and antibacterial application for spherical mesoporous Fe–N–C SAzyme. (Reproduced with permission from Ref. [78]. Copyright 2022, Elsevier). (h) ZIF-8 and its corresponding Zn–N₄ tetrahedral structural framework. (i) PMCS and the corresponding porphyrin-like structural model. (j) Photographs of *P. aeruginosa*-infected wound treated with different conditions after different lengths of time. (Reproduced with permission from Ref. [79]. Copyright 2019, Wiley-VCH).

mesoporous silicon protection strategy [79]. Systematic scrutiny of the structural evolution of SAzyme (Fig. 8h and i) uncovered its coordinatively unsaturated active Zn–N sites and elucidated its catalytic mechanism through DFT calculations. Moreover, an infected wound model involving *Pseudomonas aeruginosa* in mice was established. Treatment of wounds with PMCS and a low concentration of H₂O₂ conspicuously accelerated wound healing (Fig. 8j). This groundbreaking work is poised to propel the diversification of single-atom catalysts within the realm of biocatalysis.

4.3. Tumor therapy

Cancer, a formidable menace to human health and life, has prompted the exploration of innovative strategies for treatment. Nanozymes have emerged as a promising avenue in the pursuit of effective cancer therapy [84]. These nanozymes facilitate the transformation of specific substances within tumor cells, yielding toxic metabolites that precipitate the demise of cancerous cells [55]. The pervasive challenge of drug resistance significantly hampers the success of chemotherapy in cancer patients. Recent investigations propose a link between bacterial activity within tumors and the development of resistance by metabolizing anticancer drugs, exemplified by the nucleoside analogue gemcitabine. Gao et al. unveiled a novel anticancer strategy anchored in dual-functional N-doped carbon nanospheres (Fig. 9a) [80]. These nanospheres serve as nanoinhibitors of cytidine deaminase, overcoming gemcitabine resistance induced by bacterial activity within tumors. Notably, the nanospheres also exhibit POD-like activity, orchestrating catalytic therapy and chemotherapy within the tumor microenvironment. Their multifaceted functionality includes competitively binding to the active center of cytidine deaminase, thwarting gemcitabine metabolism by intratumoral bacteria.

Inspired by the architecture of natural enzymes, researchers have engineered SAzymes, simulating their metal–N_x active units for tumor treatment. These nanozymes respond to the tumor microenvironment by amplifying H₂O₂ as a substrate, generating ROS through Fenton reactions. Given the limited H₂O₂ concentration in tumor sites, the quest for efficient ROS production pathways is imperative. Cobalt (Co), an indispensable trace element in the human body present in vitamin B12, plays vital roles such as bone marrow red blood cell production and neuroprotection. Chen et al. devised a novel strategy for tumor catalytic therapy using Co SAzymes via cascade enzyme-catalyzed reactions (Fig. 9b). Comprising N-doped porous carbon loaded with single Co atoms (Co–SAs@NC), these nanozymes boast a large surface area, highly dispersed atomic sites, and a Co–N coordination structure. In cascade catalytic reactions, they initially emulate CAT-like activity, decomposing endogenous H₂O₂ in tumor cells to yield O₂. Subsequently, they showcase OXD-like activity, reducing O₂ to O₂^{•–}, inducing apoptosis in tumor cells. Coupled with the chemotherapy drug doxorubicin, a notable augmentation in anticancer effects was observed [81]. Zhang and colleagues synthesized a straightforward ph-CDs-Fe SAzyme using dimethyl sulfoxide as a solvent through a one-step solvothermal method [82]. Ferroin significantly increased the content of pyrrole N in ph-Fe-CDs SAzyme. Steady-state kinetic studies and DFT calculations demonstrated that ph-CDs-Fe SAzyme had higher POD-like activity than CDs and CD-Fe SAzyme. ph-CDs-Fe SAzyme, with a size of approximately 3–5 nm, exhibited excellent tumor penetration ability and the capacity to synergistically activate chemical dynamics and photothermal effects, effectively inhibiting tumor growth both *in vitro* and *in vivo* (Fig. 9c).

The tumor microenvironment is a complex milieu, housing immune cells like tumor-associated macrophages (TAMs) alongside tumor cells. The reshaping of this microenvironment using nanozymes holds promise for achieving synergistic catalytic therapy and immune regulation,

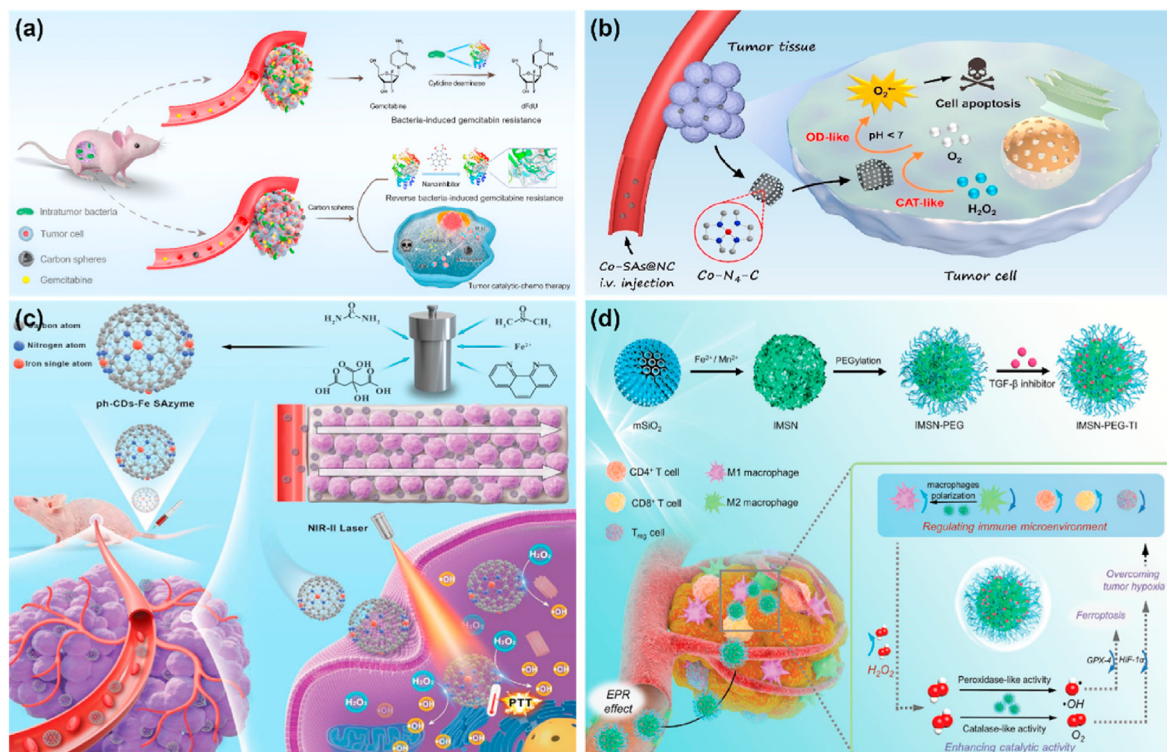


Fig. 9. (a) Schematic illustration of the strategy using carbon nanospheres to overcome tumor drug-resistance induced by intratumor bacteria and synergize gemcitabine chemotherapy with nanozyme-mediated catalytic therapy for tumor treatment. (Reproduced with permission from Ref. [80]. Copyright 2022, Wiley-VCH). (b) Illustration of nanocatalytic tumor therapy based on tumor microenvironment responsive cascade reactions by a Co SAzyme. (Reproduced with permission from Ref. [81]. Copyright 2022, Elsevier). (c) Schematic illustration of the fabrication of CDs-supported Fe SAzymes with high content of pyrrolic N and ultrasmall size (ph-CDs-Fe SAzyme) by means of a phenanthroline-mediated ligand-assisted strategy and their use to block tumor growth through synergistic chemodynamic therapy (CDT) and photothermal therapy (PTT). (Reproduced with permission from Ref. [82]. Copyright 2023, Wiley-VCH). (d) Schematic illustration of synthesis and tumor therapy for IMSN-PEG-TI. (Reproduced with permission from Ref. [83]. Copyright 2020, Wiley-VCH).

thereby enhancing the efficacy of cancer treatment. Liu and colleagues proposed a tumor catalytic therapy strategy grounded in immune microenvironment regulation, achieving efficient tumor treatment through the synergy between immune microenvironment regulation and nanozyme catalytic activity (Fig. 9d) [83]. Nanozymes exhibit dual enzyme activity, generating hydroxyl radicals with tumor-killing properties through POD-like activity and producing oxygen through catalase-like activity to alleviate tumor hypoxia. Additionally, nanozymes synergize with TGF- β inhibitors to jointly regulate the immune microenvironment, inducing polarization of M2-type macrophages to M1-type macrophages, increasing intratumoral H_2O_2 concentration, and enhancing nanozyme catalytic activity. Through the synergistic improvement of the immune microenvironment and enhanced nanozyme activity, a remarkable 87.5% tumor inhibition rate was achieved in tumor-bearing mice. This work is poised to provide fresh perspectives for the development of innovative nanozyme-based tumor catalytic therapy strategies.

4.4. Pollutant degradation

Nanozymes, harnessing a spectrum of catalytic activities, offer high stability, cost-effectiveness, and minimal environmental footprint, showcasing significant promise in the realm of environmental pollution management. Researchers have adeptly exploited the distinctive physicochemical attributes of nanomaterials, coupled with enzyme-mimicking catalytic capabilities, to proficiently craft diverse pollution control methodologies centered around nanozymes. This has led to the development of highly efficient catalytic nanozyme materials, demonstrating notable success in the removal of pollutants like phenols, anilines, and dyes.

Addressing concerns about water quality, particularly the formation of disinfection by-products during drinking water treatment, becomes imperative with the need to eliminate natural organic matter (NOM), especially humic substances (HS). As an alternative to conventional methodologies, Yu et al. present a groundbreaking study leveraging the extensive applicability of nanozymes in biocatalysis [85]. They devised a novel Fe-N-C POD-like nanozyme (FeNZ) tailored for the catalytic oxidation and degradation of NOM within a straightforward aeration process. Utilizing humic acid (HA) as a representative NOM, their findings revealed a sixfold increase in HA removal (as TOC) under low FeNZ dosage conditions (10 mg L^{-1}), compared to an aerated solution lacking FeNZ. Employing a range of analytical techniques, including cyclic voltammetry, electron spin resonance, and DFT simulations, they delved into the oxygen reduction reaction, proposing a catalytic oxidation mechanism termed "adsorption-activation-oxidation." In the FeNZ catalytic oxidation process, two forms of activated oxygen molecules, non-protonated and protonated (Fig. 10a), potentially engage in nucleophilic and electrophilic reactions with HA, respectively. Fig. 10b elucidates the intricacies of the FeNZ catalytic oxidation process. Validation results, showcasing enhanced NOM removal in natural surface water samples through FeNZ catalytic oxidation, underscore the significant potential of this oxidative enzyme-mimicking nanocatalytic material in water treatment.

Exploring the structure-activity relationship of nanozyme catalysts, single-atom catalysts emerge as a novel avenue to augment the catalytic performance of nanozymes, thanks to their maximized atomic utilization efficiency and heightened activity. Sheng et al. introduced a SAzyme catalyst featuring Fe-N₅ as the active site (Fig. 10c), deploying it in a POD-catalyzed system for the degradation of micropollutants [86]. Their investigation revealed that the Fe-N₅ SAzyme catalyst could be activated

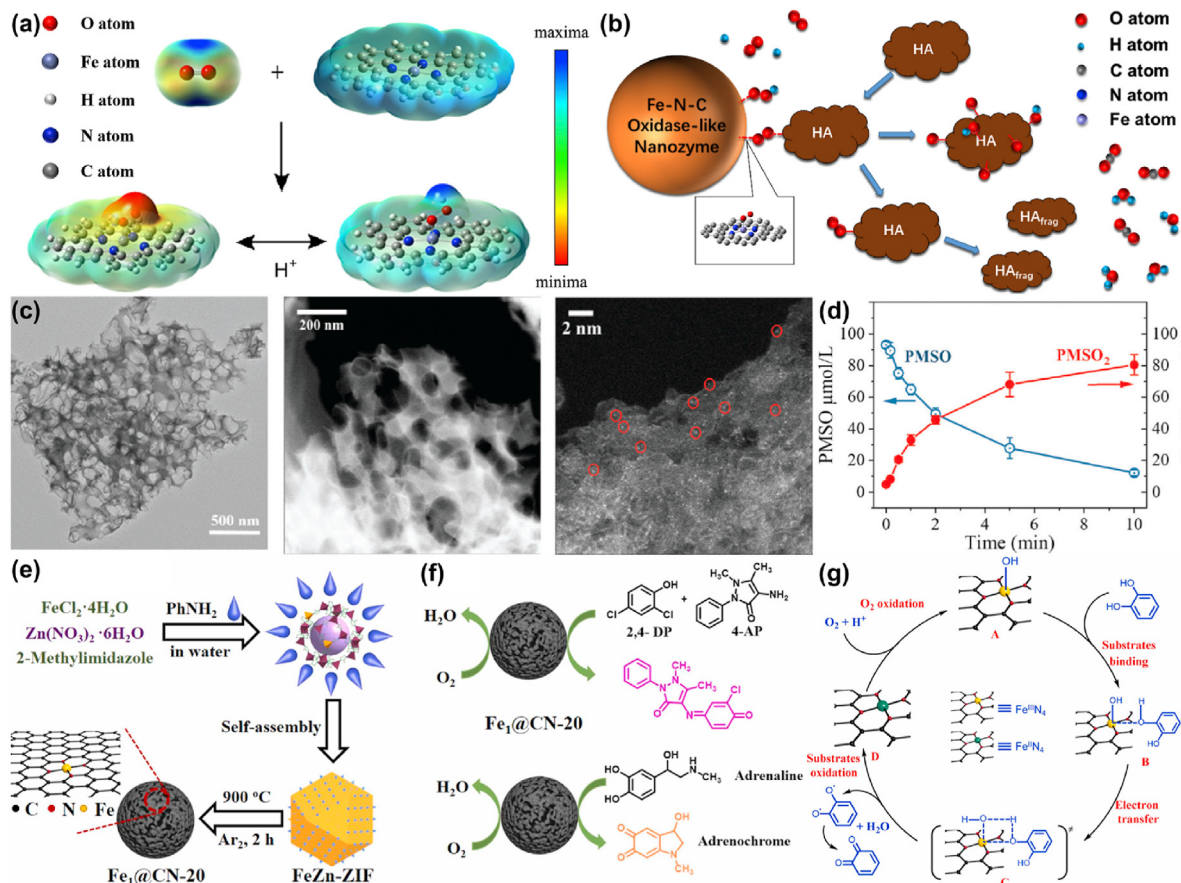


Fig. 10. (a) The optimized molecular structures and electrostatic potential surfaces of O₂, D1 site, non-protonated/protonated O₂-D1 site. (b) HA oxidation by O₂ through the catalysis of FeNZ. (Reproduced with permission from Ref. [85]. Copyright 2020, Elsevier). (c) TEM and HAADF-STEM images of Fe-SANs-800 °C. The red cycles are the single Fe atoms on the carbon substrate. (d) the kinetics of PMSO degradation and PMSO₂ production in the PMS-activated Fe-SANs system. (Reproduced with permission from Ref. [86]. Copyright 2022, Wiley-VCH). (e) Schematic diagram of Fe1@CN-20 preparation process. (f) The application of Fe1@CN-20 in detection and degradation of 2,4-DP and adrenaline. (g) The proposed catalytic mechanism for Fe1@CN-20. (Reproduced with permission from Ref. [87]. Copyright 2022, Elsevier).

by peroxides, generating Fe(IV)=O active intermediates, thereby expediting the degradation and mineralization of micropollutants (Fig. 10d). Combining experimental evidence with DFT calculations, they underscored the pivotal role of the axially coordinated N atom in the Fe-N₅ active site, providing a foundational understanding for the catalytic activity and selectivity of the nanozyme. Additionally, Lu et al. pioneered the development of an iron single-atom anchored N-doped carbon material (Fe1@CN-20) endowed with POD-like activity (Fig. 10e) [87]. The FeN₄ structure of this material adeptly mimicked POD-like activity (Fig. 10f). Despite a lower metal content compared to previous POD mimics, Fe1@CN-20 exhibited commendable stability and recyclability under extreme conditions, signaling extensive application potential for the degradation of diverse phenolic compounds (Fig. 10g).

4.5. Emerging applications

In addition to the aforementioned applications, N regulated nanozymes have also emerged with numerous novel applications [88]. Zhang et al. draw inspiration from nature and propose a novel approach utilizing cytochrome OXD-like N-C and POD-like PB for cascade reactions, achieving highly selective conversion of ascorbic acid to target products, with a selectivity up to 2000-fold higher than that of interfering reactions [89]. By employing the cascade nanozyme system in microfluidic devices, the signal is further amplified, leading to a 110-fold enhancement in sensing sensitivity (Fig. 11a-b). This study not only provides a simple and versatile method to enhance the reaction selectivity of nanozymes

but also boosts detection sensitivity. Moreover, to address more complex and demanding application scenarios, future efforts could integrate this method with other existing strategies to further optimize the selectivity of nanozyme systems. Furthermore, the study proposes a strategy to simulate higher-level biological functions of natural enzymes by endowing nanozymes with enzyme-like molecular activity centers. It is found that Fe-N-C with low-temperature carbonization exhibits markedly competitive cytochrome oxidase-like catalytic activity, contrary to ORR electrocatalytic activity, with the content and configuration of Fe-N_x sites jointly dominating the cytochrome oxidase-like catalytic process, and Fe-N-C enzyme catalysis exhibiting different electron transfer pathways from ORR catalysis (Fig. 11c) [90]. Given that P450 enzymes are involved in approximately 50% of endogenous and exogenous drug metabolism in the human body, and simultaneous intake of multiple drugs may affect P450 enzyme metabolism, leading to adverse drug reactions and even fatalities, this study further utilizes Fe-N-C to mimic the metabolism of 1,4-dihydropyridine antihypertensive drugs by P450 enzymes. Under the combined action of common antibiotics, antibiotics, or grapefruit juice, Fe-N-C exhibits inhibitory behavior similar to that of P450 enzymes.

Additionally, the overproduction of ROS can induce abnormal vascular growth, and excessive neovascularization at the retina can ultimately lead to severe retinal vascular diseases. Given the high catalytic activity of defect-engineered nanozymes in hydrogen peroxide, Fan et al. assessed their therapeutic potential for retinal neovascular diseases [91]. The study demonstrates that these nanozymes exhibit significant

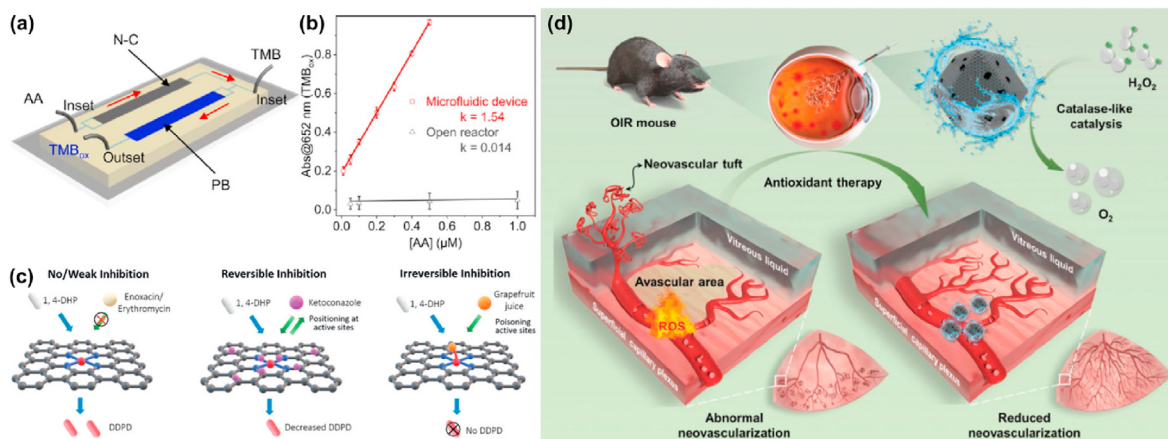


Fig. 11. (a) Cascade nanozyme reactions in a spatially confined reactor. (b) The calibration curves of oxidation and detection of AA in a microfluidic device compared to that in an open reactor. (Reproduced with permission from Ref. [89]. Copyright 2022, Wiley-VCH). (c) The possible mechanism for no/weak reversible and irreversible inhibition. (Reproduced with permission from Ref. [90]. Copyright 2020, Wiley-VCH). (d) Schematic illustration of therapeutic effects of Fe-SANzyme. The prepared Fe-SANzyme exhibits excellent catalase-like catalytic activity, which it effectively scavenges ROS, inhibits abnormal angiogenesis, and normalizes retinal neovascularization in mouse models of retinal vasculopathies. (Reproduced with permission from Ref. [91]. Copyright 2022, Wiley-VCH).

antioxidative effects in retinal endothelial cells, effectively alleviating pathological vascular dysfunction in animal models (Fig. 11d). This research not only proposes new avenues for optimizing the catalytic performance of single-atom nanozymes but also establishes a synergistic relationship between single-atom sites and adjacent defects, providing new insights into the role of defects in enzyme-catalyzed reactions within nanozyme structures. Furthermore, the development of highly active hydrogen peroxide nanozymes offers a potential therapeutic option for retinal vascular diseases.

5. Conclusions and perspectives

Compared with natural enzymes, nanozymes offer distinct advantages, characterized by their cost-effectiveness, heightened stability, exceptional durability, adjustable activity, convenient storage, and notable reusability. The catalytic performance of nanozymes is fundamentally shaped by their structural and compositional attributes. Within this review, we center our attention on the influence of N element modulation on the conceptualization and implementation of nanozymes, providing a comprehensive summary of the latest advancements in N element regulated nanozymes. Commencing with an elucidation of the foundational context and significance of nanozymes, we delve into the intricate regulatory mechanisms governed by N elements. Specific modes of N element modulation, encompassing N vacancies, N doping, N coordination, and nitrides, are systematically expounded. These regulatory pathways emerge as pivotal determinants, significantly impacting both the catalytic activity and specificity of nanozymes. The review systematically explores the pragmatic applications of N element-regulated nanozymes, spanning diverse domains such as sensing and detection, infection treatment, cancer therapy, and pollutant degradation. Concluding the discourse, the review concisely encapsulates present research revelations and outlines prospective avenues for exploration. Table S1 is dedicated to offering detailed insights into N element-regulated nanozymes for reference. Despite the strides made in understanding the regulation of nanozymes by N elements, this area of research remains in its nascent stages, presenting a landscape fraught with both opportunities and challenges as we tread into the future.

5.1. Elucidating catalytic mechanism

Through the emulation of natural enzymes, the incorporation of N elements, and the meticulous regulation of their coordination with metal

elements, a pivotal nexus is established for steering the catalytic prowess of nanozymes. This regulatory framework channels the focus of nanozyme active centers predominantly towards the intricate interplay involving N elements and the coordinating atoms in close proximity. Yet, the discernible three-dimensional architecture crafted by natural enzyme proteins casts a substantial influence on enzyme activity. The active center of natural enzymes encompasses pliable three-dimensional active cavities sculpted by specific amino acids along the peptide chain in spatial configuration. This encompasses binding centers dedicated to substrate-specific binding and centers tailored for substrate-specific catalysis. Consequently, delving into the intricacies of the N element's regulatory mechanism on nanozyme activity necessitates an expansion of the inquiry to encompass coordinating atoms neighboring N elements, and even extending the scrutiny to more distant atomic strata. Thus, a thorough exploration into the N element's regulatory mechanism on nanozyme activity mandates a holistic consideration of the interplay between N elements and the catalytic center, along with a comprehensive assessment of the overall three-dimensional structure's impact on activity. This nuanced investigation promises to furnish invaluable insights for the future design of more streamlined and efficient nanozyme catalytic systems.

5.2. Regulation catalytic specificity

In the realm of nanozymes, the recognition that a majority of these enzymes display diverse enzymatic activities under varying conditions has attracted widespread attention. This multifaceted enzymatic behavior not only facilitates synergistic interactions and mutual enhancement but also holds the potential for exhibiting detrimental and adverse effects in specific contexts. The intricacies of such multifunctionality underscore the formidable challenge of achieving a more refined regulation of the catalytic specificity inherent to nanozymes. Addressing this complex issue, the regulation of nanozymes through N elements emerges as a promising avenue. Leveraging the distinctive properties of N elements opens up possibilities for effectively fine-tuning individual or specific enzymatic activities, thereby affording nanozymes more precise and adaptable options for application. In-depth exploration in this domain not only contributes to unraveling the nuanced behavior of nanozymes within intricate catalytic networks but also introduces novel insights and methodologies for the advancement of more efficient catalytic systems.

5.3. Broadening catalytic scope

In contemporary scientific inquiry, the modulation of nanozyme activity by N elements is predominantly centered on redox enzymes, such as POD, OXD, CAT, SOD, and analogous species. Nonetheless, this scrutiny merely scratches the surface when contemplating the multifarious enzymatic functions inherent in the natural enzyme repertoire. The spectrum of catalytic activities exhibited by natural enzymes in biological organisms spans oxidation-reduction, hydrolysis, synthesis, condensation, and beyond. This underscores the imperative for an in-depth exploration into the regulation of nanozyme activity, aiming to faithfully replicate the manifold functionalities observed in natural enzymes. By judiciously engineering and overseeing the presence and distribution of N elements, the potential arises for achieving precise fine-tuning of nanozyme activity at the nanoscale. This envisioned prospect not only provides novel insights into the catalytic prowess and specificity of nanozymes but also expands their involvement in a broader array of catalytic reactions.

5.4. Synergistic catalysis effect

Certain nanozyme materials not only manifest photocatalytic and electrocatalytic attributes but also demonstrate noteworthy photo-thermal characteristics, among various other attributes. This multifaceted functionality significantly augments the efficacy of nanozymes through synergistic effects. Via photocatalysis, nanozymes can exploit light energy, converting it into chemical energy to facilitate catalytic reactions. Concurrently, their electrocatalytic properties confer superior performance in electrochemical processes, optimizing catalytic activity. Moreover, the photothermal traits of nanozymes offer an additional advantage in temperature regulation and thermosensitive catalysis. The collective interplay of these diverse properties bestows nanozymes with substantial potential for achieving synergistic effects, opening novel avenues for their utilization in energy conversion, catalytic reactions, and related domains. Through the manipulation of these composite properties, researchers can intricately design the catalytic performance of nanozymes, achieving more efficient and precisely controllable catalytic reactions. These synergistic nanozyme materials not only broaden their application scope but also provide robust support for the development of innovative catalytic systems. Subsequent research endeavors should delve deeper into elucidating the mechanisms underlying these synergistic effects to propel the widespread adoption of nanozymes across diverse scientific domains.

5.5. Investigation of toxicity

As a nanomaterial, the intricacies of nanozyme toxicity constitute a longstanding and intricate matter, influenced by a multitude of factors, with toxicity profiles varying across different types. N elements typically assume a relatively benign form within organisms, undertaking vital biological functions throughout life processes. In the modulation of nanozymes, particularly through the manipulation of N elements, meticulous consideration of their form and concentration is imperative to guarantee controlled and secure effects on organisms. Presently, nanozymes regulated by N elements exhibit potential for low toxicity, with some showcasing self-degradation capabilities under controlled modulation. Nevertheless, the intricacies of nanozyme toxicity potentially involve more sophisticated processes, encompassing how N element regulation mitigates toxicity and potential associated side effects. These aspects represent areas demanding further comprehensive research and clarification.

5.6. Expanding application areas

N element modulated nanozymes present considerable potential across a spectrum of fields encompassing sensing detection, infection

therapy, tumor therapy, and pollutant degradation. Nevertheless, the majority of applications for nanozymes remain at the prototypical stage, marked by a multitude of unresolved issues necessitating attention before practical deployment. Concurrently, domains such as anti-inflammatory and antioxidant treatments, antiviral and antifungal therapies, as well as bio-synthesis utilizing nanozymes, await comprehensive exploration of the full extent of nanozyme capabilities. Ongoing endeavors are imperative to extend the scope of applications for N element regulated nanozymes into diverse realms. Capitalizing on the distinctive attributes of N elements holds the potential to propel the advancement of nanozyme applications, ushering in novel opportunities within these domains. Subsequent research should delve deeper into elucidating the mechanisms governing N element regulated nanozymes, coupled with endeavors to optimize their performance, thus offering more practical and sustainable solutions for real-world applications.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.apmate.2024.100191>.

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