Review Article

Nano Research
2023, 16(2): 1878–1889

ISSN 1998-0124 CN 11-5974/O4
https://doi.org/10.1007/s12274-022-4856-7

Recent progress in single-atom nanozymes research

Bing Jiang, Zhanjun Guo (✉), and Minmin Liang (✉)

Experimental Center of Advanced Materials, School of Materials Science & Engineering, Beijing Institute of Technology, Beijing 100081, China

© Tsinghua University Press 2022

Received: 2 July 2022 / **Revised:** 29 July 2022 / **Accepted:** 2 August 2022

ABSTRACT

Single-atom nanozyme (SAzyme) is the hot topic of the current nanozyme research. Its intrinsic properties, such as high activity, stability, and low cost, present great substitutes to natural enzymes. Moreover, its fundamental characteristics, i.e., maximized atom utilizations and well-defined geometric and electronic structures, lead to higher catalytic activities and specificity than traditional nanozymes. SAzymes have been applied in many biomedical areas, such as anti-tumor therapy, biosensing, antibiosis, and anti-oxidation therapy. Here, we will discuss a series of representative examples of SAzymes categorized by their biomedical applications in this review. In the end, we will address the future opportunities and challenges SAzymes facing in their designs and applications.

KEYWORDS

single-atom nanozymes, natural enzymes, enzyme-like activity, biomedical applications

1 Introduction

Nanozymes are nanomaterials with intrinsic enzyme-like activity, which can efficiently catalyze the conversion of substrates and follow the similar kinetics and mechanisms of natural enzymes under physiological conditions [1]. Since the intrinsic peroxidase (POD)-like activity of Fe_3O_4 nanoparticles (NPs) was first reported in 2007 [2], many classes of nanomaterials have been reported as nanozymes which possess peroxidase-like [3, 4], oxidase-like [5, 6], superoxidase dismutase-like [7, 8], and glucose oxidase (GOx)-like [9] catalytic activities. Combining the physical and chemical properties of nanomaterials with the enzyme-like catalytic activities, nanozymes have been considered as a new generation of artificial enzymes [10]. Despite the lower cost and higher stability of nanozymes over natural enzymes, their relatively adverse activities and unclear catalytic mechanisms sometimes limit their wide applications. The situation is improved upon the emergence of single-atom nanozymes (SAzymes) owing to their high catalytic activities, fast kinetics, and definite catalytic mechanisms.

SAzymes are a class of single-atom nanomaterials (SANs) with enzyme-like activities [6]. SANs have well-defined atomic structures and electronic coordination environments leading to the unique features of maximum atomic utilizations [11–14]. Owing to those features, SAzymes, with highly assembled enzyme-like structure, show remarkable enzyme-like activities [4, 15]. Thus, SAzymes have demonstrated the potential to serve as direct alternates to traditional enzymes through direct mimics of highly evolved active sites of the natural enzymes. Moreover, SANs are at the frontier of the heterogeneous catalysts, bridging the homo- and heterogeneous catalysis with the advantages of both homogeneous catalysts (isolated active sites) and heterogeneous catalysts (stable and easy to separate) [16]. Therefore, SAzymes have attracted significant interests and been widely applied in various biomedical aspects, such as anti-tumor therapeutics, biosensing, anti-bacterial, anti-viral, anti-oxidation treatments, and others. In this review, we will focus on the recent progress of SAzymes, and summarize their significant biomedical applications from *in vitro* detection to *in vivo* monitoring and therapy.

2 Anti-tumor treatment

By catalytically generating abundant highly toxic reactive oxygen species (ROS) selectively in a tumor microenvironment, SAzymes with peroxidase, catalase-like or oxidase-like activity have been broadly exploited for antitumor therapeutics [17, 18]. Here, we describe the representative applications according to metal atoms of SAzymes, including Ru-centered, Fe-centered, Cu-centered, Co-centered, Zn-centered, Pt-centered, Pd-centered, Mn-centered et al.

In 2020, Wang et al. developed an atomically dispersed Ru SAzyme for tumor photodynamic therapy (PDT) [19]. The authors synthesized a safe and versatile self-assembled PDT nano-agent (Fig. 1(a)), OxgeMCC-r SAzyme, consisting of single-atom Ru as the active catalytic site anchored in a metal-organic framework (MOF) $Mn_3[Co(CN)_6]_2$ with encapsulated chlorin e6 (Ce6) (Figs. 1(b) and 1(c)), which served as a catalase-like nanozyme for O_2 generation (Fig. 1(d)). When conducting the *in situ* O_2 generation through the reaction between endogenous H_2O_2 and single-atom Ru species of OxgeMCC-r SAzyme, the hypoxia in tumor microenvironment was relieved leading to an enhanced ROS generation, and finally causing apoptotic cell death both *in vitro* and *in vivo*. In addition, OxgeMCC-r SAzyme showed a high loading capacity for Ce6 photosensitizer and could selectively accumulate within tumor sites for the enhanced PDT of cancer under the guide of T1 MR imaging (Figs. 1(b) and 1(d)). This study demonstrated a promising self-assembled SAzyme with highly efficient single-atom catalytic sites for cancer treatment [19]. In the same year, Shi et al. reported a bioinspired

Address correspondence to Zhanjun Guo, 6120210242@bit.edu.cn; Minmin Liang, mmliang@bit.edu.cn

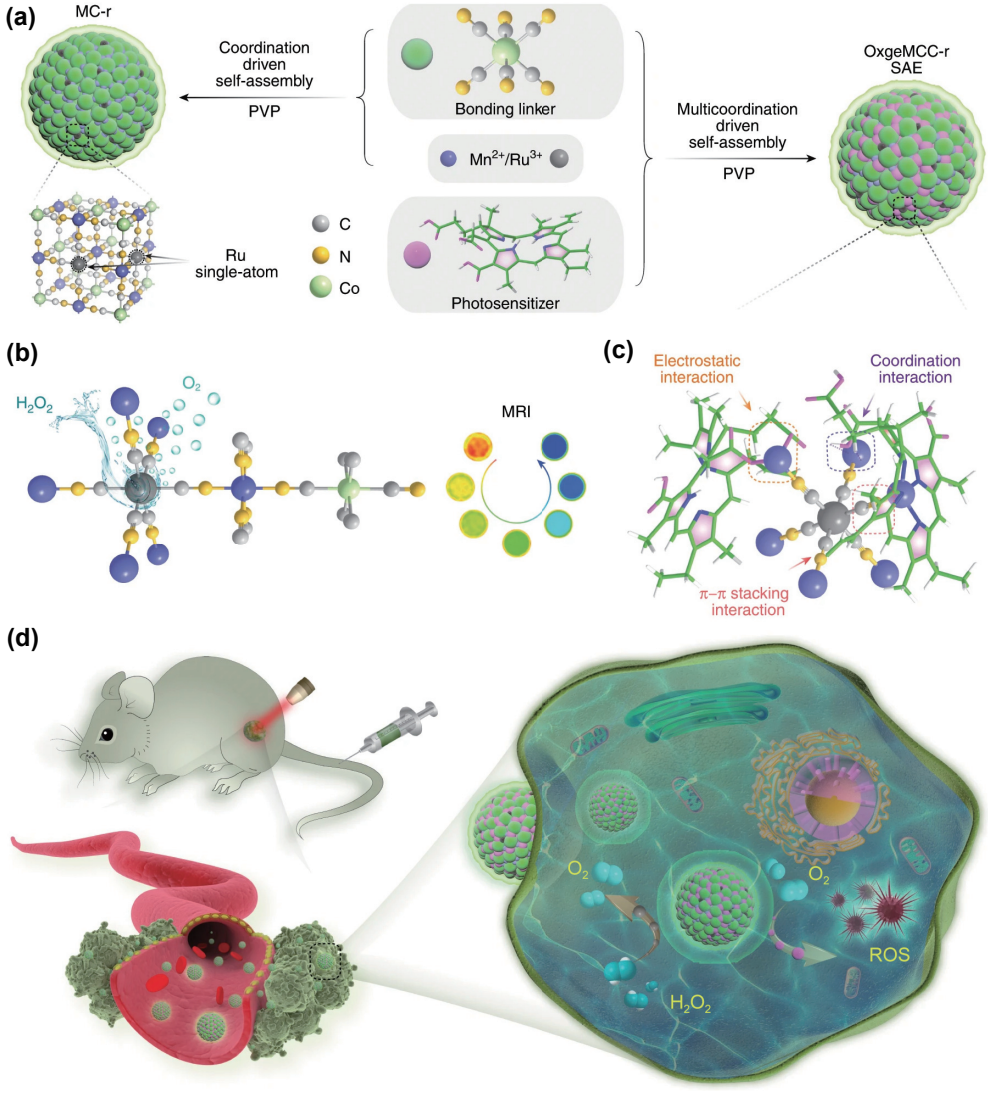

Figure 1 Design of OxgeMCC-r nanozyme with single-atom Ru for cancer treatment. (a) Schematic illustration of OxgeMCC-r, which consisted of active single-atom Ru site anchored in MCC with outer PVP protection layer. (b) Partial molecular structure of OxgeMCC-r with active single atom Ru site serving as catalase-like nanozyme for oxygen generation. (c) Multicomponent coordination interactions within the OxgeMCC-r SAzyme. (d) Scheme of continuously catalytic oxygen generation and ROS production for enhanced PDT of cancer by OxgeMCC-r SAzyme. Reproduced with permission from Ref. [19], © Wang, D. D. et al. 2020.

hollow N-doped carbon sphere doped with a single-atom copper species (Cu-HNCS), with peroxidase-like activity, that can directly catalyze the decomposition of both oxygen and hydrogen peroxide to ROS, namely superoxide ion ($O_2^{\cdot-}$) and the hydroxyl radical (·OH), respectively, in an acidic tumor microenvironment for the oxidation of intracellular biomolecules without external energy input, thus resulting in an enhanced tumor growth inhibitory effect [20]. Furthermore, in 2021, Wang et al. rationally constructed a Ru-N_2C_2 SAzyme, simultaneously acting as oxidase, peroxidase, and glutathione (GSH) oxidase mimics to synchronize the generation of ROS and the depletion of glutathione, thus amplifying the ROS damage effect, and causing the death of tumor cells [21]. And, Zhang et al. developed a novel flower-like Zn-centered carbonized nanoflowers (C-NFs) with a superior peroxidase-like activity (36.6-fold increment in the turnover frequency) that facilitates the significantly strengthened ROS generation and damage of biomolecules. To improve the water dispersion and drug encapsulation of C-NFs, apoferritin (AFt) loaded with DOX was incorporated in the interpetal space of the NFs via physical adsorption (C-NFs@D). And the following cancer cell re-sensitization is proven to be advantageous for boosting ROS-facilitated treatment of drug-resistant tumors, opening up new avenues for ROS therapy [22].

In 2021, Ji et al. reported an engineered FeN_3P SAzyme that showed the comparable peroxidase-like catalytic activity and kinetics to natural enzymes, by controlling the electronic structures of the Fe active site through the precise coordination of P and N. With its intrinsic high peroxidase-like activity, which could efficiently generate abundant oxidative species selectively under the acidic environment of tumor, the synthesized FeN_3P-SAzyme was used as an effective therapeutic strategy for inhibiting HepG2 tumor cell growth both *in vitro* and *in vivo* [3]. Similarly, Xu et al. described a rational design and controllable synthesis of FeN_5 SAzyme with superior peroxidase-like activity (Fig. 2(a)). The FeN_5 SAzyme was favorable for the generation of toxic ·OH by inducing the breakdown of H_2O_2 (Fig. 2(b)). The application of FeN_5 SAzyme achieved an effective killing of tumor cells and significantly prolonged the survival time of mice in 4T1-tumor-bearing mice model (Fig. 2(c)) [23]. Very recently, Su et al. also reported a SAzyme with single-atom Fe dispersed on N-doped mesoporous carbon nanospheres (SAFe-NMCNs). The synthesized SAFe-NMCNs SAzyme possessed dual enzyme-mimic catalytic activity which not only acted as a catalase-mimic role to achieve ultrasonic imaging in tumor site by employing O_2 generation, but also displayed the superior peroxidase-like catalytic performance which can be used to generate ·OH for tumor

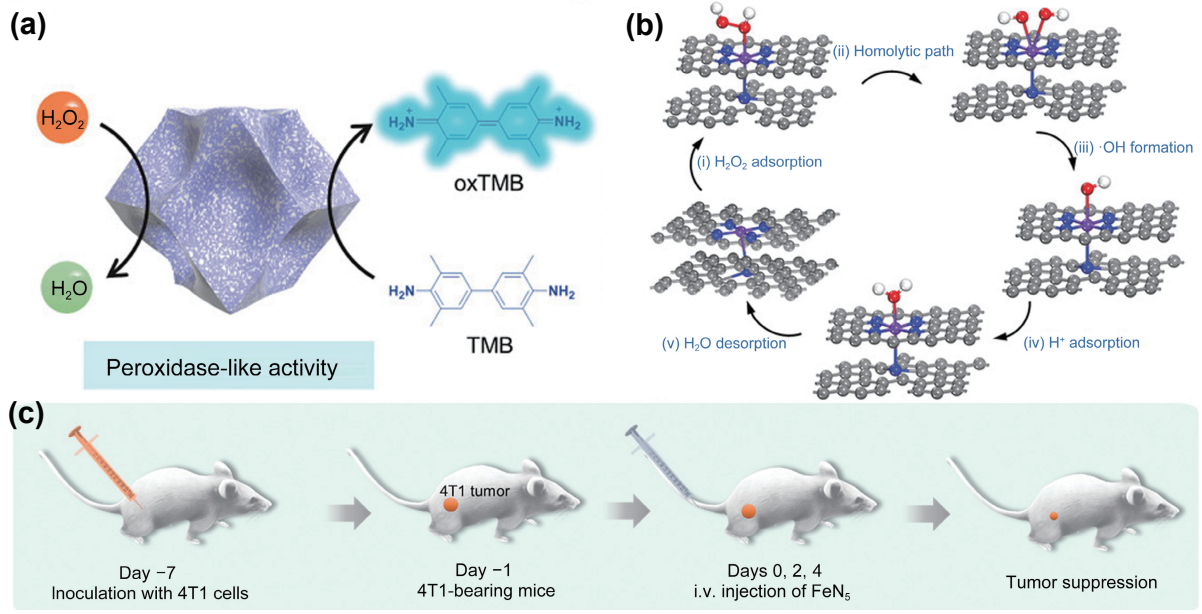

Figure 2 Tumor treatment of FeN_5 SAzyme. (a) Schematic presentation of peroxidase-like for FeN_5 SAzymes. (b) Proposed catalytic mechanism for peroxidase-like reaction on FeN_5 SAzymes. (c) Schematic illustration of therapeutic outcome of FeN_5 SAzyme administrated by i.v. injection on 4T1-tumor-bearing mice. Reproduced with permission from Ref. [23], © Wiley-VCH GmbH 2022.

therapy. In addition, SAFe-NMCNs showed strong optical absorption in the second near-infrared (NIR-II) region leading to excellent photothermal conversion performance, thus enabling the photothermal-amplified tumor catalytic therapy [24]. In 2022, Qi et al. applied a novel platelet membrane (PM)-coated mesoporous Fe SAzyme, with high peroxidase-like activity and tumor-targeting ability for tumor photothermal therapy (PTT) [25]. Apart from that, Liu et al. reported a Fe SAzyme which also could be used as a drug carrier [26]. The single-atom Fe catalytic site was capable of adsorbing and dissociating H_2O_2 specifically in tumor microenvironment, which can produce a large amount of toxic ·OH specie, resulting in a remarkable anti-tumor performance. Simultaneously, the loaded doxorubicin within Fe SAzyme could also be specifically released within tumor cells, leading to the tumor chemotherapeutic/catalytic synergistic therapy.

In 2022, Wang et al. designed and synthesized a Co/TiO_2 SAzyme with atomically dispersed Co onto the nano porous hollow TiO_2 nanoparticles using a cation-exchange strategy (Fig. 3). The Co/TiO_2 SAzyme showed both catalase-like and oxidase-like activities and could effectively converse the metabolically produced H_2O_2 in tumor microenvironment into $\cdot O_2^-$ to achieve an efficient chemodynamic therapy. Moreover, Co/TiO_2 SAzymes further exhibited the excellent biocompatibility and tolerance within biological medium, while the hollow structure facilitated the loading of drugs and imaging agents for further image-guided chemo-chemodynamic therapy via intravenous injection (Fig. 3) [27].

In 2021, Zhu et al. assembled a PEGylated Mn SAzyme by coordinating single-atom Mn to N atoms in hollow zeolitic imidazolate framework (ZIF). The developed Mn SAzymes could simultaneously convert the cellular H_2O_2 into ·OH through peroxidase-like catalyzed reaction, promote the decomposition of H_2O_2 into O_2 via catalase-like activity, and continuously catalyze the conversion of O_2 to cytotoxic $\cdot O_2^-$ via oxidase-like activity. The catalytic activity of Mn SAzymes was distinct in tumor acidic microenvironment, which enabled the sufficient generation of ROS leading to effectively killing tumor cells. In addition, the amorphous carbon of the framework possessed prominent photothermal conversion property, which can be further utilized for photothermal therapy [28].

Very recently, Chang et al. produced a Pd SAzyme using a "top-down" strategy, which focused on stripping metal NPs directly into single atoms (Fig. 4(a)). The Pd SAzymes, with the highly atom-economical utilization, simultaneously showed peroxidase-like activity, GSH oxidase-like activity, and outstanding photothermal conversion performance, which synergistically resulted in ferroptosis featuring the up-regulations of lipid peroxides (LPO) and ROS. The accumulations of LPO and ROS provided a powerful approach for cleaving heat shock proteins (HSPs), enabling Pd SAzymes mediated mild-temperature photothermal therapy (Fig. 4(b)) [29]. Likewise, Zhu et al. designed a peroxidase-like Pd SAzyme, then co-encapsulated SAzyme and camptothecin within the agarose hydrogel. In the combined SAzyme system, the Pd SAzyme converted the near-infrared laser into heat, resulting in agarose degradation and the consequent camptothecin release. Sequentially, the released camptothecin increased the H_2O_2 level within tumor cells by activating nicotinamide adenine dinucleotide phosphate oxidase, resulting in significantly increased tumor mortality [30]. In 2022, He et al. developed a phenolic Pd SAzyme with Pd single atom supported on catechol-grafted carbon-quantum-dot (DA-CQD@Pd) templates. The bio-adhesive injectable hydrogel, consisting of DA-CQD@Pd SAzymes and immune adjuvant Toll-like receptor 9 agonist CpG oligodeoxynucleotides (CpGODN), was formed through SAzyme-catalyzed free-radical polymerization. This Pd SAzyme displayed high peroxidase-like activity to generate ROS, leading to tumor cells death through catalytic therapy. In the meantime, hydrogel locally released CpGODN in a sustained manner, limiting the risk of systemic exposure and protecting CpGODN from degradations. Eventually, through its combination with the administration of immune checkpoint inhibitor anti-PD-L1, the hydrogel realized localized immunomodulation, maximized therapeutic efficacy, and prevented tumor metastasis via a catalytic immunotherapy [31].

3 Biosensing

Biosensors based on SAzymes present many remarkable merits such as ultrahigh sensitivity, high stability, and low cost [18]. At present, SAzymes have been successfully applied in colorimetric biosensing for the detections and determinations of multiple

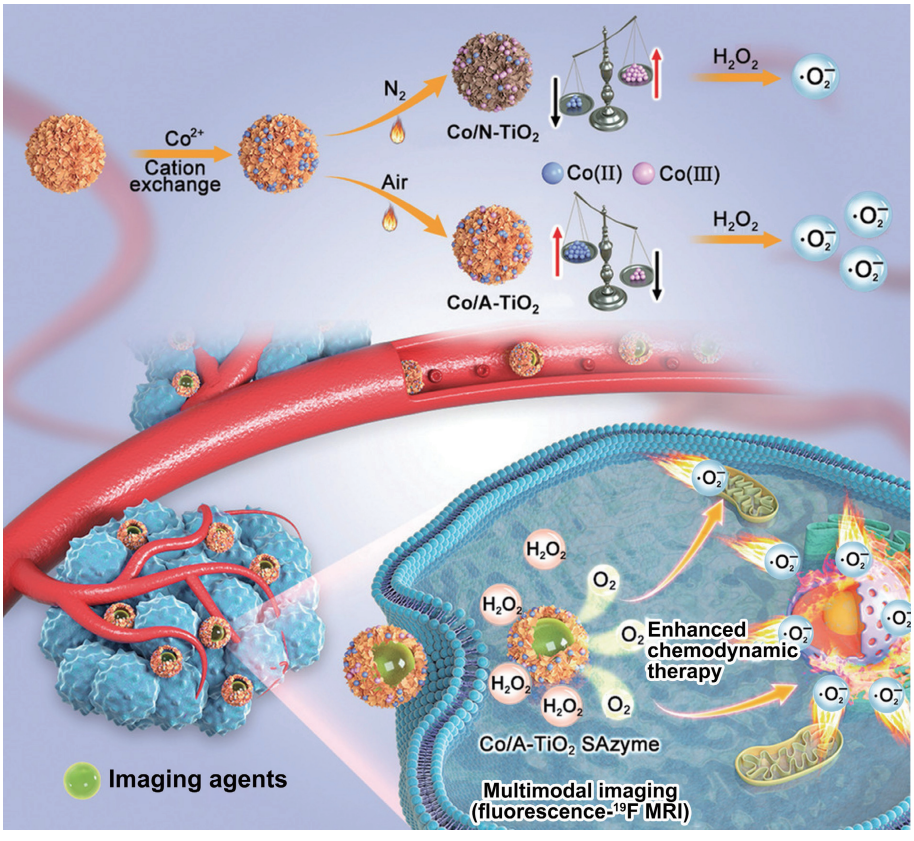

Figure 3 Illustration for the fabrication of Co/TiO_2 SAzymes with tailored activity for multimodal image-guided synergistic therapy. Reproduced with permission from Ref. [27], © Wang, H. et al. 2022.

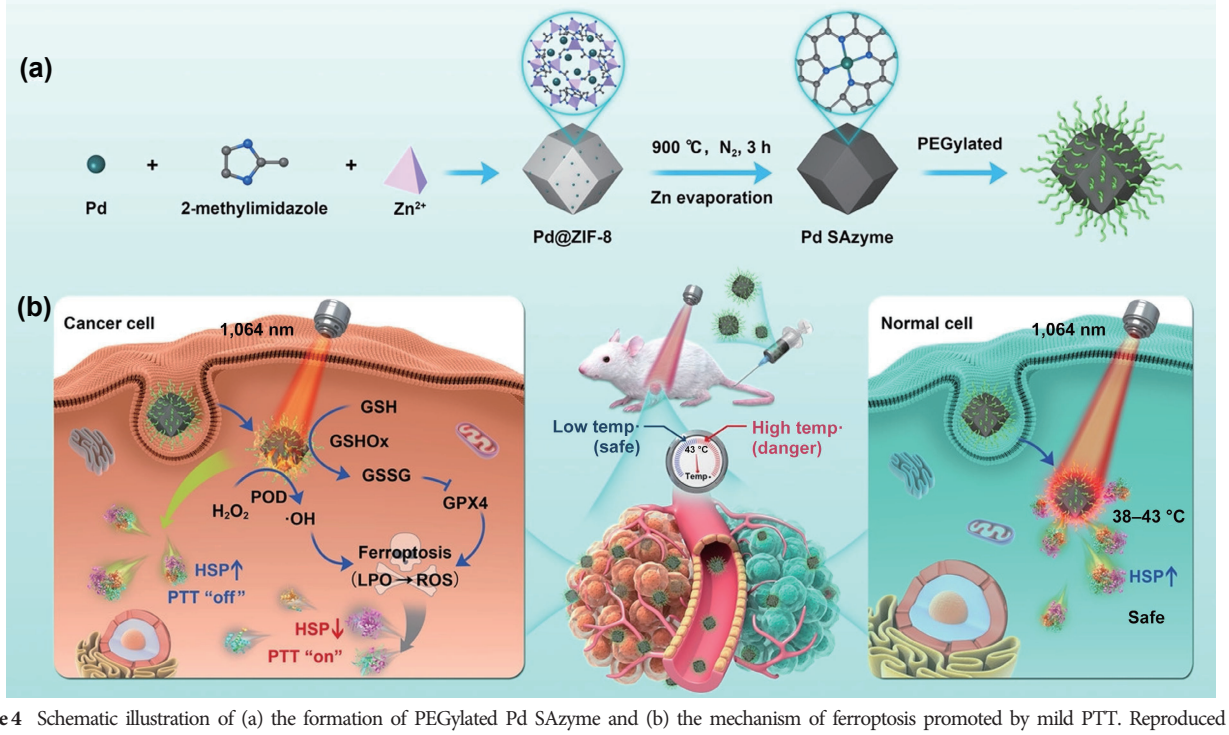

Figure 4 Schematic illustration of (a) the formation of PEGylated Pd SAzyme and (b) the mechanism of ferroptosis promoted by mild PTT. Reproduced with permission from Ref. [29], © Wiley-VCH GmbH 2021.

analytes (such as metal ions, chemical compounds, biomolecules, antigen immunoassays, and even cancer cells), and we will explain those applications thoroughly in the following sections.

In 2021, Mao et al. prepared a Fe SAzyme: SA-Fe/NG by anchoring Fe single-atom onto a single-layer of two-dimensional N-doped graphene, which enabled a fast and highly sensitive colorimetric detections of Cr(VI). The SA-Fe/NG showed superior peroxidase-like activity due to its high atomic utilization and structure. With 3,3',5,5'-tetramethylbenzidine (TMB) as a colorimetric sensing probe and 8-hydroxyquinoline (8-HQ) as an inhibitor for the oxidation of TMB, the detection of Cr(VI) was realized through specific interaction between Cr(VI) and 8-HQ, which led to the recovery of oxTMB (blue color). The newly established method showed superior sensitivities with a detection limit as low as 3 nM and has been successfully applied to the detections of Cr(VI) in tap water and tuna samples [32]. Likewise,

Li et al. designed Fe-N/S-C SAzymes, coordinating Fe by N, S, and C, which showed high oxidase-like activities. The prepared Fe-N/S-C SAzymes could oxidize the clear TMB to blue oxTMB, while the GSH can inhibit the oxidation of TMB resulting in blue color fading. When Hg^{2+} was added into the above system, Hg^{2+}-SH complexes were generated attributed to the high affinity between GSH and Hg^{2+}, ultimately leading to blue color recovery. Therefore, they created a novel "on-off-on" colorimetric sensor for the simultaneous detections of GSH (off) and Hg^{2+} (on) (Fig. 5) [33]. And in 2022, Song et al. designed and synthesized Ce-N-C SAzymes with an excellent phosphatase-like (PPA-like) activity, which could catalyze the dephosphorylation reaction of inorganic phosphates. Based on Al^{3+} high specificity towards inhibition of the PPA-like activity, they built a fast, portable, and efficient fluorescent liquid phase sensor to detect Al^{3+}. The limit of detection is as low as 22.89 ng/mL [34].

As to chemical compounds detections, in 2019, Jiao et al. designed a Fe-N-C SAzyme with excellent intrinsic peroxidase-like activity, which showed satisfactory sensitivity and specificity for colorimetric biosensing of H_2O_2 *in vitro* [35]. In 2022, Wu et al. reported a new Fe-N-C SAzyme with ultrahigh oxidase-like activity and they found out that a common analgesic-antipyretic drug 4-acetamidophenol (AMP) had inhibitory effects for the oxidase-like activity of Fe-N-C SAzymes. Therefore, they proposed a colorimetric method for AMP detection based on its inhibitory effects on Fe-N-C SAzymes, providing novel insights into investigating the interaction between nanozymes and other small molecule inhibitors [36]. In the same year, Lin et al. reported the first iron single-atom anchored on N-doped carbon material (Fe1@CN-20) as a laccase-like SAzymes, with FeN_4 structure. And they applied this SAzymes in the colorimetric sensor for a series of phenolic compounds, including 2,4-dicholorophenol, 4-chlorophenol, 2,6-dimeoxyphenol, catechol, phenol, and adrenaline [37]. In 2022, Feng et al. designed a B-doped Zn-N-C (ZnBNC) SAzyme and utilized the SAzyme as a potent peroxidase mimic. Based on the peroxidase-like activity of the ZnBNC SAzymes, a novel colorimetric method was developed for the highly sensitive (with a low detection limit: 0.1 μM) and selective detections of pphenylenediamine (PPD), which can be used in hair dyes and dyed hair samples [38]. In the same year, Li et al. constructed a Zn-N-C@hem SAzyme and developed a simple method for specific determination of both propyl gallate (PG) and formaldehyde (HCHO) by utilizing its intrinsic peroxidase-like activity to catalyze oxidation of colorless PG to yellow product. Upon addition of HCHO into the mixture of Zn-N-C@Hem and PG, the PG oxidation could be completely suppressed, resulting in notable decreases in absorbance [39].

In addition, SAzymes were also used to detect small biological molecules, including glucose, biothiols, acetylcholine, galactose, and so on. In 2021, Yan et al. reported a single-atom Bi-anchored Au (BiSA@Au) hydrogel, which showed previously unachieved POD-like activity due to the single atom Bi doping. By taking advantage of the unique porous nanowire networks of BiSA@Au hydrogels, they designed a nanozyme/natural enzyme hybrid cascade reaction system to achieve the sensitive (detection limit as low as 43.2 μM) colorimetric biosensor for glucose [40]. In 2022, Sun et al. explored the applications of the Co-N-C SAzymes, with high oxidase-like activity, as a biosensor for colorimetric detections of biothiols (GSH/Cys). The inhibitory effects of thiols toward the oxidase-like activity of Co-N-C SAzymes showed high sensitivity for GSH and Cys. Besides, the detection method showed good reproducibility and high selectivity against other amino acids [41]. Previously, in 2020, Wu et al. synthesized a peroxidase-like SAzyme with high concentration Cu sites on carbon nanosheets (Cu-N-C) through a salt-template strategy. Then, they integrated Cu-N-C SAzymes with natural acetylcholinesterase (AChE) and choline oxidase to create a three-enzyme-based cascade reaction system for the colorimetric detection of acetylcholine and organo-phosphorus pesticides [42]. In the same year, Zhou et al. developed a convenient and sensitive colorimetric strategy for the sensing of galactose based on Fe-N-C SAzymes. The Fe-SAzyme exhibited intrinsic peroxidase-like activity, which could quickly catalyze the oxidation of TMB to produce blue-colored oxTMB in the presence of H_2O_2. Since galactose can be oxidized by galactose oxidase (Gal Ox) to generate H_2O_2, Fe SAzyme can be utilized for quantitative colorimetric detections of galactose [43].

Also, SAzymes have been applied in colorimetric immunoassays. By introducing SAzymes into immunoassay, limitations of ELISA, such as low stability of HRP, can be well addressed, thereby improving its performance [15]. In 2020, Lyu et al. developed peroxidase-like Fe-N-C SAzymes derived from Fe-doped polypyrrole nanotube and substituted SAzymes for the HRP in ELISA kit to enhance the detection sensitivity of amyloid beta 1-40 (Aβ 1-40), which is a vital neuropathological hallmark for the identifications Alzheimer's disease (Fig. 6(a)) [15]. In 2021, Chen et al. synthesized highly active peroxidase-like nanozymes through loading Pt clusters on the Fe single-atom (FeSA-PtC) nanozymes. Based on its outstanding peroxidase-like activity, a cascade signal amplification strategy was constructed by

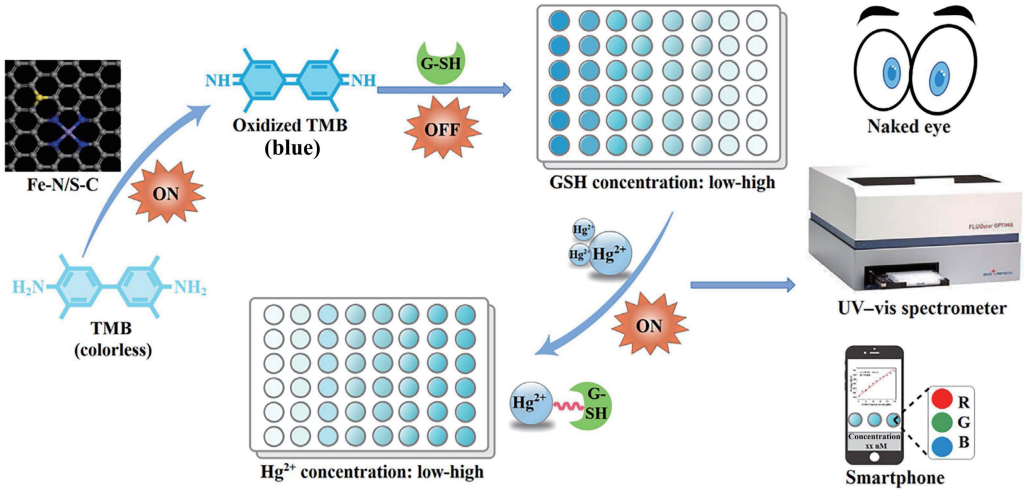

Figure 5 Schematic diagram of colorimetric sensor-based Fe-N/S-C for simultaneous multimode detections of GSH and Hg^{2+}. Reproduced with permission from Ref. [33], © Elsevier B.V. 2022.

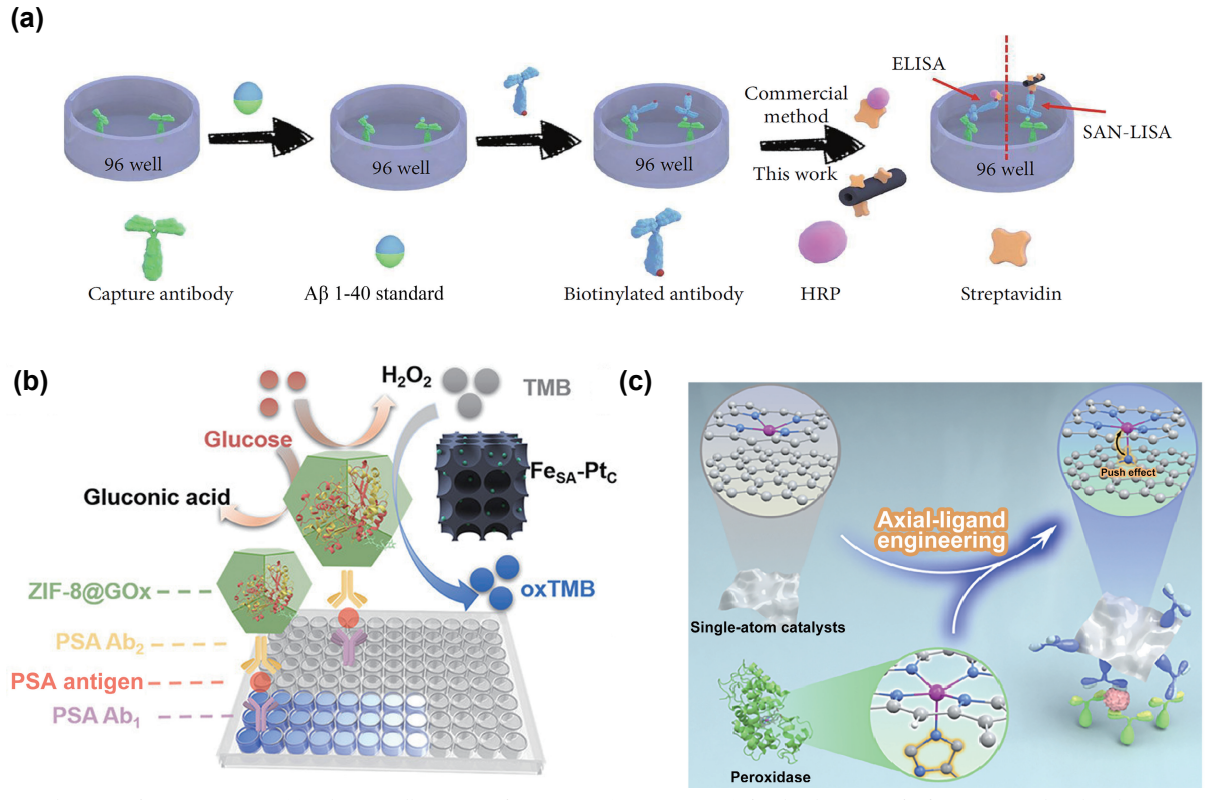

Figure 6 Schematic of SAzyme-ELISA. (a) Schematic illustration of SAN-LISA (SAzymes-LISA) for the detection of Aβ 1-40. Reproduced with permission from Ref. [15], © Zhaoyuan Lyu et al. 2020. (b) Schematic diagram of the cascade reaction for the detection of PSA by FPZ-ELISA. Reproduced with permission from Ref. [42], © American Chemical Society 2020. (c) Schematic illustration of CEA detection using the prepared SLISA. Reproduced with permission from Ref. [45], © American Chemical Society 2021.

combining GOx with FeSA-PtC nanozymes, called FeSA-PtCZIF-8/GOx ELISA (FPZ-ELISA), for the purpose of colorimetric biosensing of prostate-specific antigens (PSA). The FPZ-ELISA showed remarkable sensitivity, high selectivity, a low detection limit, and practical feasibility in serum sample detection of PSA (Fig. 6(b)) [44]. In the same year, Xu et al. designed a simple model system involving penta-coordinated and tetra-coordinated Fe-N-C single-atom catalysts (named NG-Heme and G-Heme, respectively). NG-Heme with axial ligand-engineered Fe sites exhibited superior peroxidase-like activity over G-Heme, achieving the goal of vivid mimicking of the active sites of the enzyme. Based on its exceptional catalytic activity, a NG-Heme-linked immunosorbent assay (SLISA) was constructed for colorimetric detection of carcinoembryonic antigens (CEA), with great sensitivity with a limit of detection as low as 2 pg/mL and feasibility in the analysis of clinical samples (Fig. 6(c)) [45].

In 2021, Sun et al. designed Apt/FeeNeC SAzymes (aptamer bound Fe SAzymes) for colorimetric detections of cancer cells [46]. At first, they synthesized Fe SAzymes (FeeNeC SAzymes) as the catalytic nanomaterials. Then, they engineered DNA onto SAzymes to obtain the DNA/SAzymes conjugates, which significantly improved the aqueous dispersion and recognition ability of SAzymes. More importantly, DNA modifications did not affect the peroxidase-like activity of FeeNeC SAzymes and the bioactivity of the adsorbed DNA. The Apt/FeeNeC SAzymes system took the advantage of the di-block DNA with one DNA sequence (adenine) binding to FeeNeC SAzymes and the other DNA sequence (i.e., aptamer) binding to cancer cells to detect cancer cells. This Apt/FeeNeC SAzymes system offered a new direction for the biomedicine applications of SAzymes [46].

4 Anti-biosis treatment

SAzymes also possess considerable potentials for being anti-bacterial and anti-viral applications. It is believed that the sustained production of toxic ·OH on the surface of the bacteria is one of the most feasible strategies to kill bacteria [47]. And SAzymes, as efficient antibacterial materials, exhibit high catalytic performance in promoting the generation of ·OH due to their 100% atomic utilizations and well-defined geometric and electronic structures.

In 2019, Huang et al. reported SAzymes with carbon nanoframe-confined FeN_5 active centers (FeN_5 SA/CNF), catalytically behaving like the axial ligand-coordinated heme of cytochrome P450. The electron push-effect mechanism and crucial synergistic effects endowed FeN_5 SA/CNF with high oxidase-like activity. As a result, the FeN_5 SA/CNF SAzymes showed broad-spectrum bactericidal properties *in vitro* and efficient wound disinfection characteristics *in vivo* [6]. In the same year, Xu et al. first reported a Zn-based zeolitic-imidazolate-frameworks (ZIF-8) derived carbon nanomaterial (Zn-N-C SAzyme) that could serve as a highly efficient peroxidase mimic, showing great promises for wound antibacterial applications. They found out that the inhibition rate of Zn-N-C SAC on *P. aeruginosa* was as high as 99.87%. *In vivo* infected wound model suggested that Zn-N-C SAzyme significantly promoted wound healing without considerable toxicity to various tissues and organs [48]. In 2022, Pan et al. rationally designed and synthesized a novel and vigorous chitosan grafted Fe-doped-carbon dots (CS@Fe/CDs) as an efficient artificial nanozyme to combat rigid bacterial biofilms through the selective activation of peroxidase-like catalytic activity and the synergistic antibacterial activity of chitosan. On one hand, the peroxidase-like catalytic activity of CS@Fe/CDs catalyzed the breakdown of H_2O_2 for producing ·OH, resulting in efficient cleavages of extracellular DNA (eDNA). On the other hand, chitosan was capable of binding with the negatively charged cell membrane through electrostatic interactions, changing the cell membrane permeability, and

causing cell death within bacterial biofilms. Over ~ 95.28% of *P. aeruginosa* biofilm were destroyed by the SAzyme when supplemented with H_2O_2 [49]. In 2022, spherical mesoporous Fe-N-C SAzyme was designed by Feng et al. for antibacterial therapy via photothermal treatment enhanced peroxidase-like catalytic reaction practice. The mesoporous Fe-N-C SAzyme exhibited high photothermal conversion efficiency owing to its carbon framework. And its catalytic activity could be enhanced under light irradiations due to the elevated reaction temperature. Besides that, the bacteria can also be killed via physical heat effect. Due to the synergistic effects of SAzyme catalysis and photothermal treatment, the antibacterial performance was much higher than those using single antibacterial method. *Escherichia coli* and *Staphylococcus aureus* lost 82.1% and 88.1% of its original activity, respectively, upon treating with SAzyme [50]. This work provided an alternative for combined antibacterial treatments via photothermal treatment assisted catalytic process using spherical mesoporous SAzymes as an antibiotic.

In 2021, Wang et al. constructed Cu single-atom sites/N doped porous carbon (Cu SASs/NPC) for the photothermal-catalytic antibacterial treatment by a pyrolysis-etching-adsorption-pyrolysis (PEAP) strategy. Cu SASs/NPC had strong peroxidase-like catalytic activity, GSH-depleting function, and photothermal property. Cu SASs/NPC can effectively induce peroxidase-like activity in the presence of H_2O_2, thereby generating a large amount of ·OH, which had a certain killing effect on bacteria and made bacteria more susceptible to high temperature. They introduced NIR light to generate hyperthermia to fight bacteria, and enhanced the peroxidase-like activity, thereby generating additional ·OH to destroy bacteria. In addition, Cu SASs/NPC can act as GSH peroxidase (GSH-Px)-like nanozymes, which can deplete GSH in bacteria, to significantly improve the sterilization effect. They found out the PTT-catalytic synergistic antibacterial strategy showed almost 100% antibacterial efficiency against *E. coli* and methicillin-resistant *S. aureus* (MRSA) (Fig. 7(a)). *In vivo* experiments also indicated a better PTT-catalytic synergistic therapeutic performance on MRSA-infected mouse wounds (Fig. 7(b)) [51].

In 2022, Fan et al. synthesized a SAzyme based on Pt single atoms modified carbon nitride nanorod (SA-Pt/g-C_3N_4-K), which exhibited peroxidase-like activity for antibacterial applications. Both experimental results and density functional theory (DFT) calculations revealed that the Pt-N-C structure of SA-Pt/g-C_3N_4-K significantly enhanced ·OH generation during H_2O_2 activation. And due to its intrinsic peroxidase-like activity, the applications of SA-Pt/g-C_3N_4-K showed remarkable hydroxyl radical-mediated *in vitro* Gram-negative bacteria inactivation performance with killing efficiency > 99.99% in the presence of H_2O_2 and enabled healing

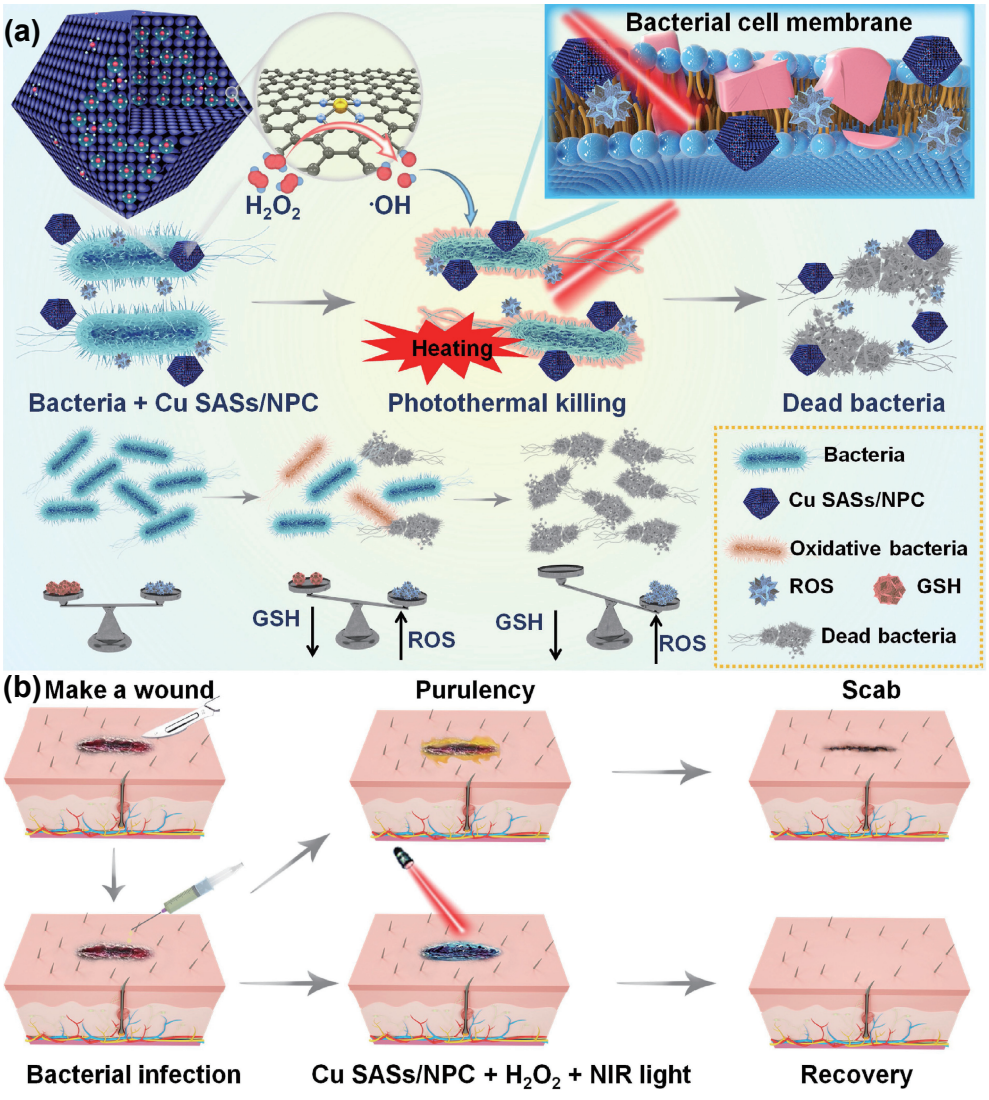

Figure 7 Cu SASs/NPC with GSH-depleting performance were synthesized for photothermal-catalytic therapy against bacterial. (a) Cu SASs/NPC as GSH peroxidase-like mimetic enzyme and peroxidase-like nanozyme for eradicating *E. coli* and MRSA *in vitro*, (b) and for the treatment of MRSA infection *in vivo*. Reproduced with permission from Ref. [51], © Wang, X. W. et al. 2021.

of Gram-negative bacteria-infected wounds [52]. In the same year, Yu et al. prepared an injectable ointment using MOF-based single-atom catalysis systems with good biocompatibility and high anti-biofilm activity, consisting of PEG-400/PEG-4000 and Pt single-atom doped porphyrin metal-organic framework (PCN-222-Pt). The PCN-222-Pt SAzymes showed strong peroxidase-like and oxidase-like activities, so it could produce reactive oxygen species spontaneously, indicating an excellent anti-biofilm performance *in vitro* (98.69% against *S. aureus* biofilm, 99.91% against *E. coli* biofilm). Besides, they found out that the injectable PCN-222-Pt ointment demonstrated alleviative inflammation responses in the treatment of biofilm-induced periodontitis, leading to reduced bone destruction and healthier gum tissue, which is superior to the current clinically widely used Periocline [53].

Additionally in 2021, Wang et al. designed a TiO_2 supported single-atom nanozyme containing atomically dispersed Ag atoms (Ag-TiO_2 SAN) to serve as a highly efficient antiviral nanomaterial. Ag-TiO_2 SAN exhibited higher adsorption (99.65%) of SARS-CoV2 pseudo-virus, due to the interactions between the SAN and spike 1 protein of receptor binding domain (RBD) of SARS-CoV2. The theoretical calculations and experimental evidence indicated that the Ag atom of the SAN strongly bound to Cys and Asp, which are the most abundant amino acids on the surface of the spike 1 RBD. After binding to the virus, the SAN/virus complex was typically phagocytosed by macrophages and colocalized with lysosomes. In addition, Ag-TiO_2 SAN showed high peroxidase-like activity which was responsible for ROS production under the microenvironment of lysosomes (i.e., acid conditions), favoring the oxygen reduction reaction process to eliminate the SARS-CoV2. Finally, from the viral elimination models, Ag-TiO_2 SAN displayed an efficient anti-SARS-CoV2 pseudo-virus activity. In conclusion, they successfully designed and achieved a novel high adsorption and peroxidase-like SAzymes for resisting and clearing SARS-CoV2 (Fig. 8) [52].

5 Anti-oxidation therapy

Excessive ROS or reactive nitrogen species (RNS/RONS) can result in severe oxidative damages to biomolecules (such as nucleic acids and proteins) and induce cell apoptosis and inflammations. SAzymes have been shown as promising candidates in oxidative stress cytoprotecting applications by clearing out ROS and RNS inside the cells [18].

In 2019, Yan et al. developed a nanozyme-based bandage using single-atom Pt/CeO_2 with persistent catalytic activities (peroxidase-like, catalase-like, glutathione peroxidase (GPx)-like, and superoxide dismutase (SOD)-like activities) for noninvasive treatments of neurotrauma (Fig. 9). They found out that Pt/CeO_2 SAzymes had great RNS, which is closely related to brain trauma, scavenging abilities. And the Pt/CeO_2 SAzymes-based bandage also provided sustained catalytic activities. Both *in vitro* and *in vivo* results showed that the bandage could decrease indicators of oxidative stress and inflammation responses of neuron cells and improve impaired neurocognition. Their work provided a promising cutting-edge example for noninvasive treatment of traumatic brain injury using SAzymes [55]. In the same year, Lu et al. found out that Fe-N/C SAzymes, which they synthesized in previous study, exhibited the properties of various mimic enzymes, including peroxidase-like, oxidase-like, catalase-like, and GPx-like activity. Based on its intrinsic properties of catalase-like and GPx-like activities, they established that Fe-N/C SAzymes could effectively protect cells from oxidative stress by scavenging intracellular ROS [56]. In 2020, Cao et al. reported a novel SAzyme, Co/PMCS, featuring atomically dispersed coordinatively unsaturated active Co-porphyrin centers. The Co/PMCS can be used as an efficient antioxidant therapy to alleviate sepsis, due to its ability to fast obliterate multiple RONS. Also, Co/PMCS eliminated $\cdot O_2^-$ and H_2O_2 by mimicking superoxide dismutase, catalase, and glutathione peroxidase activity, while removing ·OH via the oxidative-reduction cycle, exhibiting significantly higher activity. In addition, it can also scavenge ·NO through the

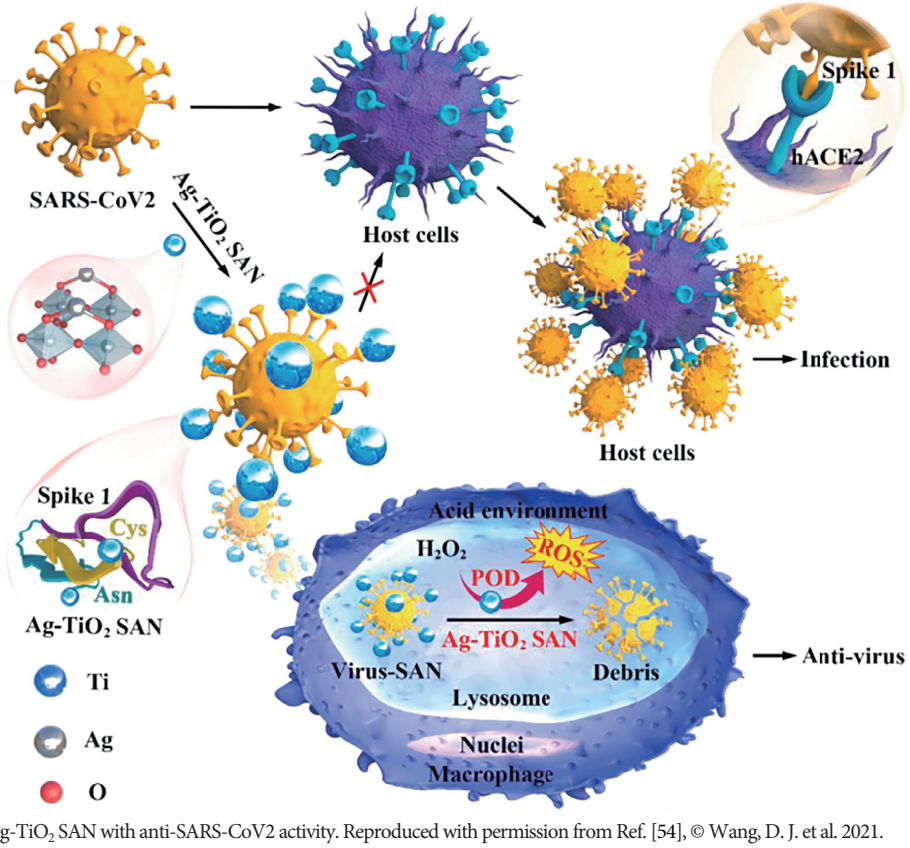

Figure 8 Schematic of Ag-TiO_2 SAN with anti-SARS-CoV2 activity. Reproduced with permission from Ref. [54], © Wang, D. J. et al. 2021.

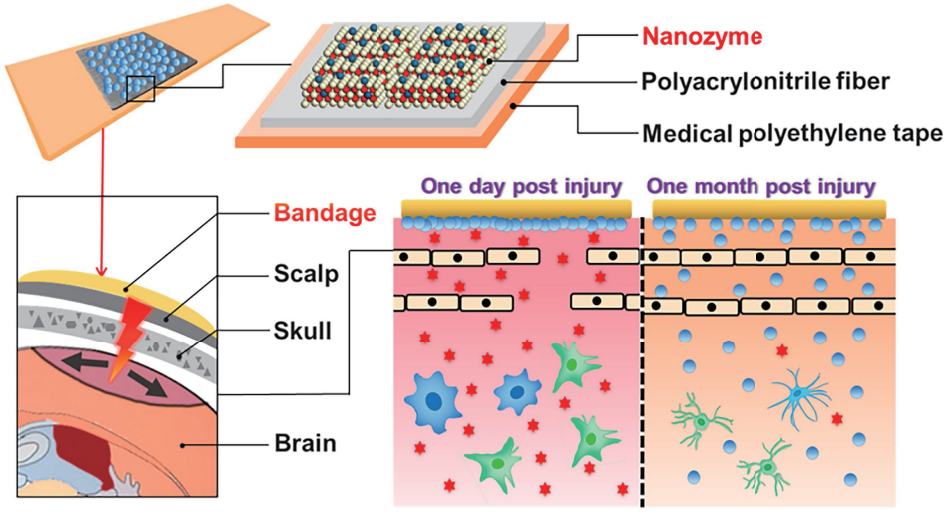

Figure 9 Concept of using the Pt/CeO_2 single-atom nanozymes for treatment of brain trauma. Reproduced with permission from Ref. [55], © American Chemical Society 2019.

formation of nitrosyl-metal complex. And they found out that Co/PMCS could reduce proinflammatory cytokine level, protect organs from damage, and confer a distinct survival advantage to the infected sepsis mice, demonstrating its application as a promising agent for antioxidative therapy [57]. In 2021, Liu et al. designed artificial $Au_{24}Cd_1$ and $Au_{24}Cu_1$ SAzymes based on a structurally well-defined Au_{25} cluster using a delicate single-atom substitution approach. They found out that $Au_{24}Cu_1$ and $Au_{24}Cd_1$ showed compelling selectivity in glutathione catalase-like and superoxide dismutase-like activities, respectively. And $Au_{24}Cu_1$ could decrease the peroxide concentration in injured brain via catalytic reaction process, while $Au_{24}Cd_1$ preferentially utilized superoxide and nitrogenous signal molecules as substrates and significantly decreased inflammation factors, indicative of an important role in mitigating neuroinflammation [58]. In 2022, Chen et al. designed Cu SAs/CN with isolated single Cu atoms anchored on a graphitic carbon nitride (g-C_3N_4), which showed intrinsic ascorbate peroxidase (APX)-like behaviors with comparable specific catalytic activity and kinetics to the natural APXs. From the DFT calculations, they learned that Cu-N_4 moieties in the active center of Cu SAs/CN were determined to leading to such favorable APX catalytic performance, in which the electron transfer between Cu and coordinated N atoms facilitated the activation and cleavage of the adsorbed H_2O_2 molecules and resulted in fast kinetics. The constructed Cu SAs/CN nanozyme with superior APX-like performance and high biocompatibility could be applied for effectively protecting the H_2O_2-treated cells against oxidative injury *in vitro* [59].

6 A special single-atom nanozyme: Dual active site

Except for the general SAzymes with one kind of dispersed metal atom, the dual-atom nanozymes (DAzymes) were established with two kinds of metal atoms coordinated with the surrounding supports [18, 60]. DAzymes are double-atom nanomaterials (DANs) with enzyme-like activity. DANs emerge as a novel frontier in heterogeneous catalysis, because the synergistic effects between adjacent active sites can promote their catalytic activity while maintaining high atomic utilization efficiency, good selectivity, and high stability originating from the atomically dispersed nature [60]. DAzymes are more than simple doubling of single-atom and they may introduce a synergistic effect to break the theoretical limits of SAzymes. Also, it can provide a new strategy for the mimic of natural enzymes which sometimes have two kinds of metal atoms as the active sites, (for example: Cu-Zn SOD).

In 2020, Du et al. engineered FeCu-DA/NC DAzymes with Fe-Cu dual atomic sites embedded in three-dimensional porous N-doped carbon, mimicking both the constituents of active centers and enzymatic microenvironment of cytochrome c oxidase (CcO). The FeCu-DA/NC exhibited a superior oxidation reduction reaction (ORR) activity in both acid and base conditions. And the DFT calculations further proved that the performance can be attributed to the unique synergistic effect between Cu and Fe single atoms in which Cu-N_4 served as the electron donor to increase the electron density of the active centers, Fe-N_4, thus facilitating O_2 activation [61]. In 2022, Zhao et al. designed a paradigm of synergy and "division of labor" bimetallic non-alloy structured atom pair (FeCo-DIA/NC), combining ROS circulation with parallel catalytic therapy for efficient tumor therapy (Fig. 10(a)). FeCo-DIA/NC synergistically initiated both peroxidase-like and oxidase-like catalytic reactions, and FeCo-DIA/NC showed higher activity than single-atom Fe-SIA/NC or single-atom Co-SIA/NC. In addition, FeCo-DIA/NC could directly and simultaneously catalyze H_2O_2 and O_2 into ROS (·OH, $\cdot O_2^-$, and $^1O_2$). The above ROS were further converted into H_2O_2 in the acid tumor microenvironment, forming a "ROS cycle" for highly efficient tumor inhibition (Fig. 10(b)) [62]. In 2022, Chen et al. reported a novel Fe-Bi bimetallic MOF-derived carbon supported Fe-N_4 and Bi-N_4 dual-site FeBi-NC DAzymes for cascade catalysis and peroxymonosulfate (PMS) activation to degrade dye pollutants (rhodamine B). The FeBi-NC DAzymes had high single atoms loadings of Fe and Bi and displayed 5.9- and 9.8-fold oxidase-like activity enhancement relative to the Fe-NC and Bi-NC SAzymes, respectively. Then, they integrated AChE with FeBi-NC DAzymes, to develop a cascade enzyme-nanozyme system for selective and sensitive screening of AChE activity. Also, FeBi-NC displayed a strong binding energy and electron donating capability to generate highly active intermediates for rhodamine B degradation [63]. In the same year, Ma et al. presented a facile route towards synthesizing a DAzyme containing Zn/Mo dual single-atom catalyst supported on the macroscope aerogel (Zn/Mo DSAC-SMA), which utilized the non-covalent nano-assembly of polyoxometalates, supramolecular coordination complexes as the metal-atom precursor. The DAzyme of Zn and Mo had a high content and exhibited a synergistic effect and remarkable peroxidase-like activity, with 3-fold increase relative to Zn/Mo DSAC-SMA. The Zn/Mo DSAC-SMA was applied in the detection of versatile analytes, including intracellular H_2O_2, glucose in serum, cholesterol, and ascorbic acid [64].

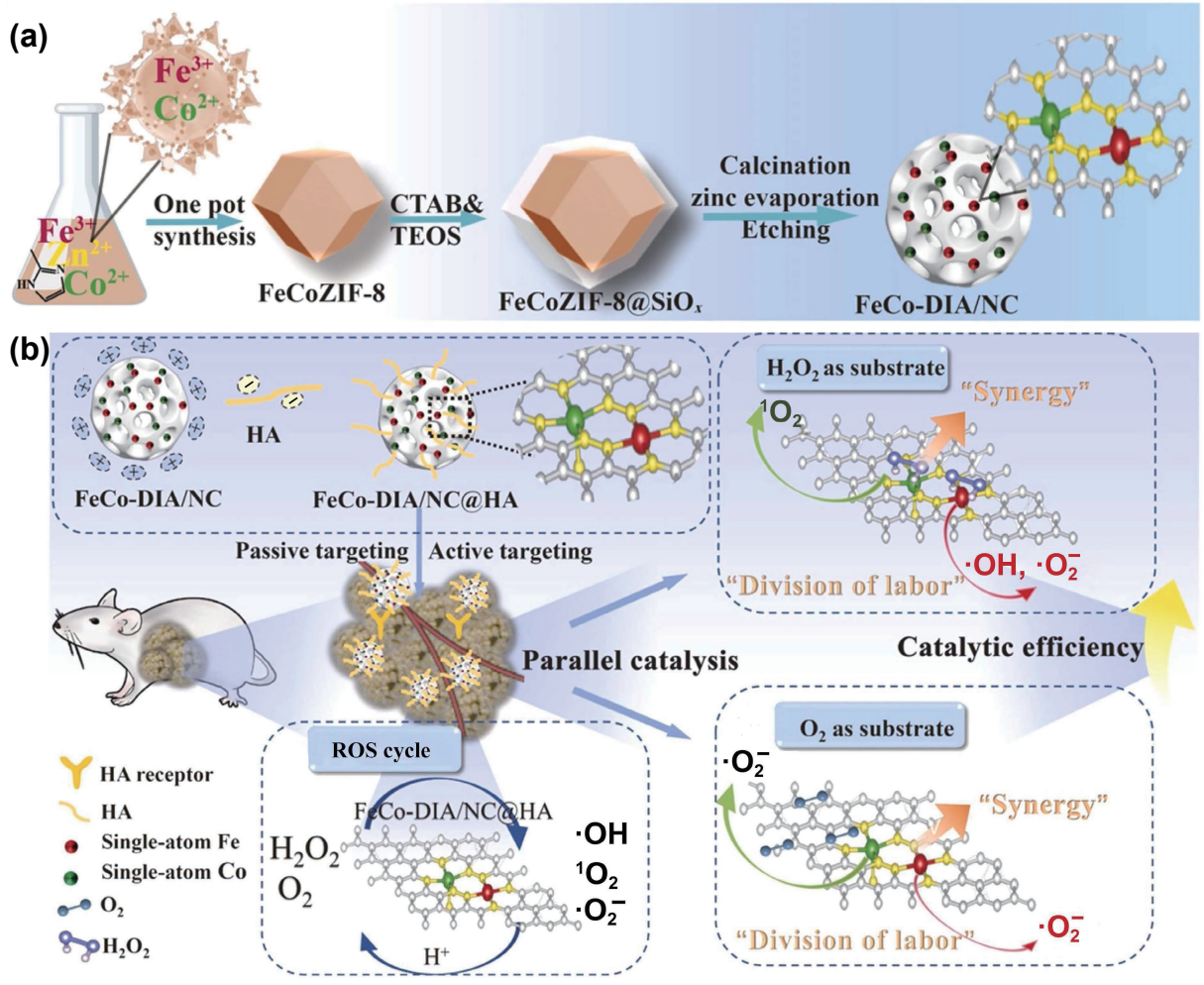

Figure 10 (a) Synthetic schematic diagram of FeCo-DIA/NC. (b) Catalytic mechanism for the synergy and "division of labor" of FeCo-DIA/NC for tumor ROS cycle and parallel catalytic therapy. Reproduced with permission from Ref. [62], © Elsevier Ltd. 2022.

7 Summary and perspective

Single-atom nanozymes are the research frontiers of nanozymes, which are the next-generation artificial enzymes, in recent five years. As the single-atom catalysts with intrinsic enzyme-like characteristics, SAzymes have the advantages such as low cost, high stability, ease to mass production, and multiple functionalities compared with natural enzymes and conventional artificial enzymes. In addition, SAzymes with atomically dispersed active sites possess unique geometrical and electronic structures, unsaturated coordination environments, and highly dispersed metal atoms doped on various supports, which exhibit superior performances in catalytic activity and selectivity. All those advantages provide SAzymes with more possibilities for rational designs and broader applications. However, the designs of SAzymes with enough catalytic activity and selectivity still have a long way to go, to meet the demands of biomedical applications. Although great opportunities lie ahead for the developments of SAzymes, there are still some remaining challenges needing to be addressed too.

(1) The enhancement of catalytic activity, selectivity, and specificity.

Promoting catalytic activities has always been an important research direction for nanozymes. Although SAzymes have generally high catalytic activities owing to their dispersed single atoms and well-defined electronic and geometric structures, few can match with or even better than natural enzymes. And SAzymes often have multienzyme-like activities. To address these matters, researchers should take advantage of coenzyme factor coordination (e.g., Heme), heteroatoms doping (such as P and S), coordination number regulations, and other methods to regulate the electronic structures of SAzymes to further improve the specificity and activity of SAzymes. Besides, the presence of dual-atom nanoenzymes also provides a new strategy to increase SAzymes activities, because of the unique synergistic effect between two different single atoms to increase the electron density of the active centers.

(2) Expanding the types of SAzymes.

At present, most activities of SAzymes are oxidoreductases, such as peroxidase-like, oxidase-like, catalase-like, and SOD-like activities. It leads us to wonder whether new types of enzyme-like activities can be identified for the SAzymes. As mentioned above, Fe SAzymes can in principle mimic the structure and catalytic performance of natural enzymes (e.g., Fe-N_4 mimic HRP and Fe-N_5 mimic cytochrome P450). In a similar way, researchers could potentially mimic other natural enzymes (e.g., laccase, oxidase, glutathione peroxidase and superoxide dismutase, and cytochrome c oxidase) by further making use of the information from the enzymes' active sites, such as atomic structures, oxidation states, coordination chemistry, and others. In addition, DAzymes also give researchers the chance to simulate enzymes containing two mental centers in their active sites.

(3) Biosafety of SAzymes.

As we mentioned earlier, SAzymes have been applied in many biomedical areas, such as anti-tumor, anti-biosis, and anti-oxidation therapies. And in the biomedical research, biocompatibility and biodegradability are the two biologically critical indicators for biosafety. However, most of present studies

are focusing on SAzymes' enzyme-like catalytic performances, their biosafety has not been investigated thoroughly. When SAzymes enter cells, the metallic elements and organic or inorganic supports might be dangerous to normal cells and tissues. Therefore, the SAzymes' pharmacokinetics and dose-related toxicology, which related to biocompatibility and biodegradability, should be studied and improved. To help solve this problem, researchers should optimize the surface bioconjugation chemistry of SAzymes, such as using amino acids, DNA, and polysaccharide modifications. In addition, the elemental choices of the metal center atoms and the supports should be screened for the safer combinations. Besides, researchers should always take advantage of the localized physiological environment variables, such as the pH difference, redox levels, or hypoxic conditions between normal tissues and diseased sites, which impact the metabolic profiles and cytotoxicity of SAzymes.

Acknowledgements

This work was supported by the National Key Research and Development (R&D) Program of China (No. 2017YFA0205501), the National Natural Science Foundation of China (Nos. T2225026 and 82172087), the Youth Innovation Promotion Association of Chinese Academy of Sciences (No. 2018017), and the China Postdoctoral Science Foundation (No. 2021M690383).

References

[1] Gao, L. Z.; Yan, X. Y. Nanozymes: An emerging field bridging nanotechnology and biology. *Sci. China Life Sci.* **2016**, *59*, 400–402.

[2] Gao, L. Z.; Zhuang, J.; Nie, L.; Zhang, J. B.; Zhang, Y.; Gu, N.; Wang, T. H.; Feng, J.; Yang, D. L.; Perrett, S. et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat. Nanotechnol.* **2007**, *2*, 577–583.

[3] Ji, S. F.; Jiang, B.; Hao, H. G.; Chen, Y. J.; Dong, J. C.; Mao, Y.; Zhang, Z. D.; Gao, R.; Chen, W. X.; Zhang, R. F. et al. Matching the kinetics of natural enzymes with a single-atom iron nanozyme. *Nat. Catal.* **2021**, *4*, 407–417.

[4] Jiang, B.; Duan, D. M.; Gao, L. Z.; Zhou, M. J.; Fan, K. L.; Tang, Y.; Xi, J. Q.; Bi, Y. H.; Tong, Z.; Gao, G. F. et al. Standardized assays for determining the catalytic activity and kinetics of peroxidase-like nanozymes. *Nat. Protoc.* **2018**, *13*, 1506–1520.

[5] Vernekar, A. A.; Das, T.; Ghosh, S.; Mugesh, G. A remarkably efficient $MnFe_2O_4$-based oxidase nanozyme. *Chem. Asian J.* **2016**, *11*, 72–76.

[6] Huang, L.; Chen, J. X.; Gan, L. F.; Wang, J.; Dong, S. J. Single-atom nanozymes. *Sci. Adv.* **2019**, *5*, eaav5490.

[7] Yang, M.; Jiang, W.; Pan, Z. Q.; Zhou, H. Synthesis, characterization and SOD-like activity of histidine immobilized silica nanoparticles. *J. Inorg. Organomet. Polym. Mater.* **2015**, *25*, 1289–1297.

[8] Heckert, E. G.; Karakoti, A. S.; Seal, S.; Self, W. T. The role of cerium redox state in the SOD mimetic activity of nanoceria. *Biomaterials* **2008**, *29*, 2705–2709.

[9] Periasamy, A. P.; Roy, P.; Wu, W. P.; Huang, Y. H.; Chang, H. T. Glucose oxidase and horseradish peroxidase like activities of cuprous oxide/polypyrrole composites. *Electrochim. Acta* **2016**, *215*, 253–260.

[10] Wei, H.; Wang, E. K. Nanomaterials with enzyme-like characteristics (nanozymes): Next-generation artificial enzymes. *Chem. Soc. Rev.* **2013**, *42*, 6060–6093.

[11] Chen, Y. J.; Wang, P. X.; Hao, H. G.; Hong, J. J.; Li, H. J.; Ji, S. F.; Li, A.; Gao, R.; Dong, J. C.; Han, X. D. et al. Thermal atomization of platinum nanoparticles into single atoms: An effective strategy for engineering high-performance nanozymes. *J. Am. Chem. Soc.* **2021**, *143*, 18643–18651.

[12] Zhu, P.; Xiong, X.; Wang, D. S. Regulations of active moiety in single atom catalysts for electrochemical hydrogen evolution reaction. *Nano Res.* **2022**, *15*, 5792–5815.

[13] Li, R. Z.; Wang, D. S. Understanding the structure–performance relationship of active sites at atomic scale. *Nano Res.* **2022**, *15*, 6888–6923.

[14] Zheng, X. B.; Li, B. B.; Wang, Q. S.; Wang, D. S.; Li, Y. D. Emerging low-nuclearity supported metal catalysts with atomic level precision for efficient heterogeneous catalysis. *Nano Res.*, in press, https://doi.org/10.1007/s12274-022-4429-9.

[15] Lyu, Z. Y.; Ding, S. C.; Zhang, N.; Zhou, Y.; Cheng, N.; Wang, M. Y.; Xu, M. J.; Feng, Z. X.; Niu, X. H.; Cheng, Y. et al. Single-atom nanozymes linked immunosorbent assay for sensitive detection of Aβ 1-40: A biomarker of Alzheimer's disease. *Research* **2020**, *2020*, 4724505.

[16] Chen, F.; Jiang, X. Z.; Zhang, L. L.; Lang, R.; Qiao, B. T. Single-atom catalysis: Bridging the homo- and heterogeneous catalysis. *Chin. J. Catal.* **2018**, *39*, 893–898.

[17] Pei, J. H.; Zhao, R. L.; Mu, X. Y.; Wang, J. Y.; Liu, C. L.; Zhang, X. D. Single-atom nanozymes for biological applications. *Biomater. Sci.* **2020**, *8*, 6428–6441.

[18] Shi, Q. L.; Yu, T. R.; Wu, R. F.; Liu, J. Metal–support interactions of single-atom catalysts for biomedical applications. *ACS Appl. Mater. Interfaces* **2021**, *13*, 60815–60836.

[19] Wang, D. D.; Wu, H. H.; Phua, S. Z. F.; Yang, G. B.; Lim, W. Q.; Gu, L.; Qian, C.; Wang, H. B.; Guo, Z.; Chen, H. Z. et al. Self-assembled single-atom nanozyme for enhanced photodynamic therapy treatment of tumor. *Nat. Commun.* **2020**, *11*, 357.

[20] Lu, X. Y.; Gao, S. S.; Lin, H.; Yu, L. D.; Han, Y. H.; Zhu, P.; Bao, W. C.; Yao, H. L.; Chen, Y.; Shi, J. L. Bioinspired copper single-atom catalysts for tumor parallel catalytic therapy. *Adv. Mater.* **2020**, *32*, 2002246.

[21] Wang, W. Y.; Zhu, Y.; Zhu, X. R.; Zhao, Y. F.; Xue, Z. G.; Xiong, C.; Wang, Z. Y.; Qu, Y. T.; Cheng, J. J.; Chen, M. et al. Biocompatible ruthenium single-atom catalyst for cascade enzyme-mimicking therapy. *ACS Appl. Mater. Interfaces* **2021**, *13*, 45269–45278.

[22] Xing, Y. X.; Wang, L.; Wang, L. C.; Huang, J. X.; Wang, S.; Xie, X. Y.; Zhu, J.; Ding, T.; Cai, K. Y.; Zhang, J. X. Flower-like nanozymes with large accessibility of single atom catalysis sites for ROS generation boosted tumor therapy. *Adv. Funct. Mater.* **2022**, *32*, 2111171.

[23] Xu, B. L.; Li, S. S.; Zheng, L. R.; Liu, Y. H.; Han, A.; Zhang, J.; Huang, J. J.; Xie, H. J.; Fan, K. L.; Gao, L. Z. et al. A bioinspired five-coordinated single-atom iron nanozyme for tumor catalytic therapy. *Adv. Mater.* **2022**, *34*, 2107088.

[24] Su, Y. T.; Wu, F.; Song, Q. X.; Wu, M. J.; Mohammadniaei, M.; Zhang, T. W.; Liu, B. L.; Wu, S. S.; Zhang, M.; Li, A. et al. Dual enzyme-mimic nanozyme based on single-atom construction strategy for photothermal-augmented nanocatalytic therapy in the second near-infrared biowindow. *Biomaterials* **2022**, *281*, 121325.

[25] Qi, P. Y.; Zhang, J. Y.; Bao, Z. R.; Liao, Y. P.; Liu, Z. M.; Wang, J. K. A platelet-mimicking single-atom nanozyme for mitochondrial damage-mediated mild-temperature photothermal therapy. *ACS Appl. Mater. Interfaces* **2022**, *14*, 19081–19090.

[26] Liu, Y.; Yao, M.; Han, W. X.; Zhang, H. R.; Zhang, S. S. Construction of a single-atom nanozyme for enhanced chemodynamic therapy and chemotherapy. *Chem.—Eur. J.* **2021**, *27*, 13418–13425.

[27] Wang, H.; Wang, Y.; Lu, L. L.; Ma, Q.; Feng, R. X.; Xu, S. Y.; James, T. D.; Wang, L. Y. Reducing valence states of Co active sites in a single-atom nanozyme for boosted tumor therapy. *Adv. Funct. Mater.* **2022**, *32*, 2200331.

[28] Zhu, Y.; Wang, W. Y.; Cheng, J. J.; Qu, Y. T.; Dai, Y.; Liu, M. M.; Yu, J. N.; Wang, C. M.; Wang, H. J.; Wang, S. C. et al. Stimuli-responsive manganese single-atom nanozyme for tumor therapy via integrated cascade reactions. *Angew. Chem., Int. Ed.* **2021**, *60*, 9480–9488.

[29] Chang, M. Y.; Hou, Z. Y.; Wang, M.; Yang, C. Z.; Wang, R. F.; Li, F.; Liu, D. L.; Peng, T. L.; Li, C. X.; Lin, J. Single-atom Pd nanozyme for ferroptosis-boosted mild-temperature photothermal therapy. *Angew. Chem., Int. Ed.* **2021**, *60*, 12971–12979.

[30] Zhu, D. M.; Chen, H.; Huang, C. Y.; Li, G. X.; Wang, X.; Jiang, W.; Fan, K. L. H_2O_2 self-producing single-atom nanozyme hydrogels as

light-controlled oxidative stress amplifier for enhanced synergistic therapy by transforming "cold" tumors. *Adv. Funct. Mater.* **2022**, *32*, 2110268.

[31] He, H.; Fei, Z. Y.; Guo, T. L.; Hou, Y.; Li, D.; Wang, K. F.; Ren, F. Z.; Fan, K. L.; Zhou, D. J.; Xie, C. M. Bioadhesive injectable hydrogel with phenolic carbon quantum dot supported Pd single atom nanozymes as a localized immunomodulation niche for cancer catalytic immunotherapy. *Biomaterials* **2022**, *280*, 121272.

[32] Mao, Y.; Gao, S. J.; Yao, L. L.; Wang, L.; Qu, H.; Wu, Y. E.; Chen, Y.; Zheng, L. Single-atom nanozyme enabled fast and highly sensitive colorimetric detection of Cr(VI). *J. Hazard. Mater.* **2021**, *408*, 124898.

[33] Li, R.; He, X. T.; Javed, R.; Cai, J.; Cao, H. M.; Liu, X.; Chen, Q.; Ye, D. X.; Zhao, H. B. Switching on-off-on colorimetric sensor based on Fe-N/S-C single-atom nanozyme for ultrasensitive and multimodal detection of Hg^{2+}. *Sci. Total Environ.* **2022**, *834*, 155428.

[34] Song, G. C.; Li, J. C.; Majid, Z.; Xu, W. T.; He, X. Y.; Yao, Z. Y.; Luo, Y. B.; Huang, K. L.; Cheng, N. Phosphatase-like activity of single-atom Ce-N-C nanozyme for rapid detection of Al^{3+}. *Food Chem.* **2022**, *390*, 133127.

[35] Jiao, L.; Xu, W. Q.; Yan, H. Y.; Wu, Y.; Liu, C. R.; Du, D.; Lin, Y. H.; Zhu, C. Z. Fe-N-C single-atom nanozymes for the intracellular hydrogen peroxide detection. *Anal. Chem.* **2019**, *91*, 11994–11999.

[36] Wu, W. W.; Huang, L.; Zhu, X. Y.; Chen, J. X.; Chao, D. Y.; Li, M. H.; Wu, S. L.; Dong, S. J. Reversible inhibition of the oxidase-like activity of Fe single-atom nanozymes for drug detection. *Chem. Sci.* **2022**, *13*, 4566–4572.

[37] Lin, Y. M.; Wang, F.; Yu, J.; Zhang, X.; Lu, G. P. Iron single-atom anchored N-doped carbon as a "laccase-like" nanozyme for the degradation and detection of phenolic pollutants and adrenaline. *J. Hazard. Mater.* **2022**, *425*, 127763.

[38] Feng, M.; Zhang, Q.; Chen, X. F.; Deng, D.; Xie, X. Y.; Yang, X. P. Controllable synthesis of boron-doped Zn-N-C single-atom nanozymes for the ultrasensitive colorimetric detection of p-phenylenediamine. *Biosens. Bioelectron.* **2022**, *210*, 114294.

[39] Li, H.; Li, Q. L.; Shi, Q.; Wang, Y. J.; Liu, X. W.; Tian, H.; Wang, X. R.; Yang, D. Z.; Yang, Y. L. Hemin loaded Zn-N-C single-atom nanozymes for assay of propyl gallate and formaldehyde in food samples. *Food Chem.* **2022**, *389*, 132985.

[40] Yan, H. Y.; Jiao, L.; Wang, H. J.; Zhu, Y. M.; Chen, Y. F.; Shuai, L.; Gu, M.; Qiu, M.; Gu, W. L.; Zhu, C. Z. Single-atom Bi-anchored Au hydrogels with specifically boosted peroxidase-like activity for cascade catalysis and sensing. *Sensor. Actuat. B: Chem.* **2021**, *343*, 130108.

[41] Sun, L. P.; Yan, Y.; Chen, S.; Zhou, Z. J.; Tao, W.; Li, C.; Feng, Y.; Wang, F. Co-N-C single-atom nanozymes with oxidase-like activity for highly sensitive detection of biothiols. *Anal. Bioanal. Chem.* **2022**, *414*, 1857–1865.

[42] Wu, Y.; Wu, J. B.; Jiao, L.; Xu, W. Q.; Wang, H. J.; Wei, X. Q.; Gu, W. L.; Ren, G. X.; Zhang, N.; Zhang, Q. H. et al. Cascade reaction system integrating single-atom nanozymes with abundant Cu sites for enhanced biosensing. *Anal. Chem.* **2020**, *92*, 3373–3379.

[43] Zhou, X. B.; Wang, M. K.; Chen, J. Y.; Xie, X. L.; Su, X. G. Peroxidase-like activity of Fe-N-C single-atom nanozyme based colorimetric detection of galactose. *Anal. Chim. Acta* **2020**, *1128*, 72–79.

[44] Chen, Y. F.; Jiao, L.; Yan, H. Y.; Xu, W. Q.; Wu, Y.; Zheng, L. R.; Gu, W. L.; Zhu, C. Z. Fe-N-C single-atom catalyst coupling with Pt clusters boosts peroxidase-like activity for cascade-amplified colorimetric immunoassay. *Anal. Chem.* **2021**, *93*, 12353–12359.

[45] Xu, W. Q.; Song, W. Y.; Kang, Y. K.; Jiao, L.; Wu, Y.; Chen, Y. F.; Cai, X. L.; Zheng, L. R.; Gu, W. L.; Zhu, C. Z. Axial ligand-engineered single-atom catalysts with boosted enzyme-like activity for sensitive immunoassay. *Anal. Chem.* **2021**, *93*, 12758–12766.

[46] Sun, L. P.; Li, C.; Yan, Y.; Yu, Y.; Zhao, H.; Zhou, Z. J.; Wang, F.; Feng, Y. Engineering DNA/Fe-N-C single-atom nanozymes interface for colorimetric biosensing of cancer cells. *Anal. Chim. Acta* **2021**, *1180*, 338856.

[47] Xiang, H. J.; Feng, W.; Chen, Y. Single-atom catalysts in catalytic biomedicine. *Adv. Mater.* **2020**, *32*, 1905994.

[48] Xu, B. L.; Wang, H.; Wang, W. W.; Gao, L. Z.; Li, S. S.; Pan, X. T.; Wang, H. Y.; Yang, H. L.; Meng, X. Q.; Wu, Q. W. et al. A single-atom nanozyme for wound disinfection applications. *Angew. Chem., Int. Ed.* **2019**, *58*, 4911–4916.

[49] Pan, T.; Chen, H. H.; Gao, X.; Wu, Z. Y.; Ye, Y. W.; Shen, Y. Z. Engineering efficient artificial nanozyme based on chitosan grafted Fe-doped-carbon dots for bacteria biofilm eradication. *J. Hazard. Mater.* **2022**, *435*, 128996.

[50] Feng, Y. Y.; Qin, J.; Zhou, Y.; Yue, Q.; Wei, J. Spherical mesoporous Fe-N-C single-atom nanozyme for photothermal and catalytic synergistic antibacterial therapy. *J. Colloid Interface Sci.* **2022**, *606*, 826–836.

[51] Wang, X. W.; Shi, Q. Q.; Zha, Z. B.; Zhu, D. D.; Zheng, L. R.; Shi, L. X.; Wei, X. W.; Lian, L.; Wu, K. L.; Cheng, L. Copper single-atom catalysts with photothermal performance and enhanced nanozyme activity for bacteria-infected wound therapy. *Bioact. Mater.* **2021**, *6*, 4389–4401.

[52] Fan, Y. F.; Gan, X. R.; Zhao, H. M.; Zeng, Z. X.; You, W. J.; Quan, X. Multiple application of SAzyme based on carbon nitride nanorod-supported Pt single-atom for H_2O_2 detection, antibiotic detection and antibacterial therapy. *Chem. Eng. J.* **2022**, *427*, 131572.

[53] Yu, Y.; Cheng, Y.; Tan, L.; Liu, X. M.; Li, Z. Y.; Zheng, Y. F.; Wu, T.; Liang, Y. Q.; Cui, Z. D.; Zhu, S. L. et al. Theory-screened MOF-based single-atom catalysts for facile and effective therapy of biofilm-induced periodontitis. *Chem. Eng. J.* **2022**, *431*, 133279.

[54] Wang, D. J.; Zhang, B.; Ding, H.; Liu, D.; Xiang, J. Q.; Gao, X. J.; Chen, X. H.; Li, Z. J.; Yang, L.; Duan, H. X. et al. TiO_2 supported single Ag atoms nanozyme for elimination of SARS-CoV2. *Nano Today* **2021**, *40*, 101243.

[55] Yan, R. J.; Sun, S.; Yang, J.; Long, W.; Wang, J. Y.; Mu, X. Y.; Li, Q. F.; Hao, W. T.; Zhang, S. F.; Liu, H. L. et al. Nanozyme-based bandage with single-atom catalysis for brain trauma. *ACS Nano* **2019**, *13*, 11552–11560.

[56] Lu, M. J.; Wang, C.; Ding, Y. Q.; Peng, M. H.; Zhang, W.; Li, K.; Wei, W.; Lin, Y. Q. Fe-N/C single-atom catalysts exhibiting multienzyme activity and ROS scavenging ability in cells. *Chem. Commun.* **2019**, *55*, 14534–14537.

[57] Cao, F. F.; Zhang, L.; You, Y. W.; Zheng, L. R.; Ren, J. S.; Qu, X. G. An enzyme-mimicking single-atom catalyst as an efficient multiple reactive oxygen and nitrogen species scavenger for sepsis management. *Angew. Chem., Int. Ed.* **2020**, *59*, 5108–5115.

[58] Liu, H. L.; Li, Y. H.; Sun, S.; Xin, Q.; Liu, S. H.; Mu, X. Y.; Yuan, X.; Chen, K.; Wang, H.; Varga, K. et al. Catalytically potent and selective clusterzymes for modulation of neuroinflammation through single-atom substitutions. *Nat. Commun.* **2021**, *12*, 114.

[59] Chen, Y.; Zou, H.; Yan, B.; Wu, X. J.; Cao, W. W.; Qian, Y. H.; Zheng, L.; Yang, G. W. Atomically dispersed Cu nanozyme with intensive ascorbate peroxidase mimic activity capable of alleviating ROS-mediated oxidation damage. *Adv. Sci.* **2022**, *9*, 2103977.

[60] Ying, Y. R.; Luo, X.; Qiao, J. L.; Huang, H. T. "More is different": Synergistic effect and structural engineering in double-atom catalysts. *Adv. Funct. Mater.* **2021**, *31*, 2007423.

[61] Du, C.; Gao, Y. J.; Chen, H. Q.; Li, P.; Zhu, S. Y.; Wang, J. G.; He, Q. G.; Chen, W. A Cu and Fe dual-atom nanozyme mimicking cytochrome c oxidase to boost the oxygen reduction reaction. *J. Mater. Chem. A* **2020**, *8*, 16994–17001.

[62] Zhao, M. Y.; Yang, R. G.; Wei, Y. R.; Su, J. J.; Wang, X. N.; Zhang, N.; Sun, P. C.; Chen, D. L.; Zhao, Y. X. Dual isolated bimetal single-atom catalysts for tumor ROS cycle and parallel catalytic therapy. *Nano Today* **2022**, *44*, 101493.

[63] Chen, Q. M.; Liu, Y.; Lu, Y. W.; Hou, Y. J.; Zhang, X. D.; Shi, W. B.; Huang, Y. M. Atomically dispersed Fe/Bi dual active sites single-atom nanozymes for cascade catalysis and peroxymonosulfate activation to degrade dyes. *J. Hazard. Mater.* **2022**, *422*, 126929.

[64] Ma, C. B.; Xu, Y. P.; Wu, L. X.; Wang, Q.; Zheng, J. J.; Ren, G. X.; Wang, X. Y.; Gao, X. F.; Zhou, M.; Wang, M. et al. Guided synthesis of a Mo/Zn dual single-atom nanozyme with synergistic effect and peroxidase-like activity. *Angew. Chem., Int. Ed.* **2022**, *61*, e202116170.