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ARTICLE

Single-atom nanozymes as promising catalysts for biosensing and biomedical applications

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Nanozymes with intrinsic enzyme-like properties and excellent stability are promising alternatives to natural enzymes. Yet, the low density of active sites and unclear crystal structure has been the major obstacles that impede the progress. Single-atom nanozymes (SAzymes) have emerged as a unique system to mitigate these issues, due to maximal atomic utilization, well-defined electronic and geometric structures, and outstanding catalytic activity distinct from their nanosized counterparts. Furthermore, the homogeneously dispersed active sites and well-defined coordination structures provide rare pathways to shed light onto the catalytic mechanisms. In this review, we will summarize the latest progress in the rational design and engineering of SAzymes and their applications in biomedicine and biosensing. We will conclude the review with highlights of the remaining challenges and perspectives of this emerging technology.

Contents

1. Introduction

Enzymes are biocatalysts produced by living cells that possess a high catalytic activity promoting specific biological reactions in the body and maintain cell life activities. Most enzymes are proteins (a small number are RNA), and their catalytic activities can be greatly impacted by the chemical environment, as the enzymes are prone to degradation, and the high cost of preparation renders it difficult for large-scale production. To mitigate these issues, artificial enzymes have been attracting extensive attention as the stable and low-cost alternatives.[1, 2] In 2007, Yan et al. found that Fe_3O_4 nanoparticles exhibited peroxidase (POD)-like activity and the optimal catalytic conditions were approximate to those of natural horseradish peroxidase (HRP), which was a significant breakthrough in the field of nanomaterials biocatalysis.[3] Since then, the term "nanozyme" has attracted widespread attention,[4-6] due largely to the high stability, low cost, tuneable catalytic activity, and ease of large-scale production.[7, 8] There are three major types of nanozymes based on their substrates, carbon-based nanozymes, metal-based nanozymes, and metal oxide-based

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nanozymes,[9] featuring catalytic activities resembling those of natural enzymes like POD, oxidase (OXD), catalase (CAT), superoxide dismutase (SOD), glucose oxidase (GOx) and hydrolase.[10-12] Thus far, nanozymes have been widely adopted in diverse fields, such as disease diagnosis and treatment, biosensing, food safety, environmental monitoring, chemical production, among others.[13-15]

Whereas nanozymes may possess properties that are superior to those of natural enzymes, the catalytic activity and substrates affinity remain mostly subpar as compared to that of natural enzymes. This is mainly attributed to the low density of active sites in nanozymes, and the unclear crystal structure and complex elemental composition make it difficult to unravel the catalytic mechanisms.[16, 17] To overcome these barriers, single-atom nanozymes (SAzymes) have emerged as a unique platform, where the catalytic activity can be enhanced by the maximal utilization of metal atoms and the well-defined atomic configuration offers unprecedented insights into the mechanistic origin.[18]

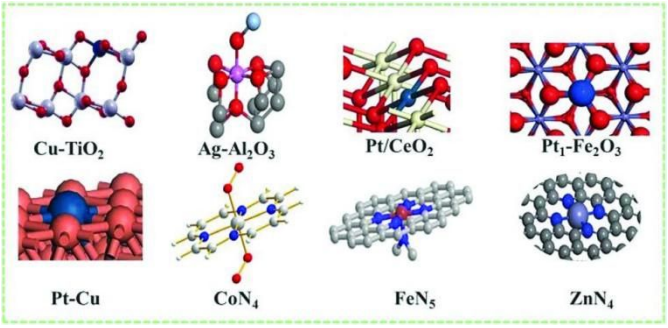
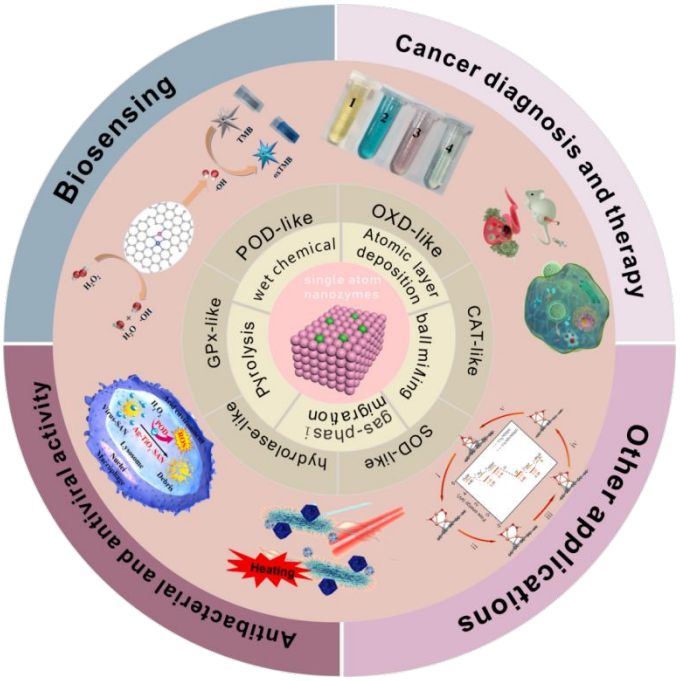


Figure 1. Representative SAzymes with metal centers homogeneously dispersed within a range of supporting scaffolds, such as carbon, metal oxides, and metals. Reproduced with permission from ref. 20, Copyright 2020, Royal Society of Chemistry.

In SAzymes, isolated metal atoms are anchored onto a select structural scaffold and the resultant coordination moiety serves as the active centers (**Figure 1**).[19, 20] SAzymes combine the advantages of homogeneous catalysts and heterogeneous catalysts and may break the limitations in material design for unprecedented catalytic activity.[21, 22] In most studies, SAzymes exhibit POD- and OXD-like activity,[23-25] and can break down H_2O_2 to reactive oxygen molecules (ROS) that are potent agents in the inhibition of the growth of harmful cells such as bacteria and viruses.[26-28] Meanwhile, by deliberate manipulation of the coordination structure between the metal atoms and the supports, the SAzyme active centers can be carefully tailored, leading to multienzyme catalytic activity for enzyme cascade reactions,[29, 30] and a catalytic performance competitive to that of the natural enzyme.[31] Meanwhile, the well-defined active sites and electronic properties render it possible to reveal the structure-activity correlation.[32-34] In addition, the development of effective synthetic strategies, such as wet impregnation and high-temperature annealing, have made it possible for large-scale preparation of SAzymes with a high metal coverage and stable metal coordination structure, as compared to conventional synthesis methods.[35] The structural insights can also be exploited for the construction of relevant models for theoretical calculations where further insights into the mechanistic origin can be obtained.[36]



Scheme 1. Schematic illustration of SAzymes for a broad scope of biologically critical applications.

In this review, we will summarize the latest progress in the design and synthesis of SAzymes and their applications in biomedicines and biosensing, highlight the remaining challenges and put forward opportunities in future research (**Scheme 1**).

2. Synthetic Strategies

Experimentally, a range of structural parameters, in particular, the coordination configuration of the metal centers and the geometric structure of the supporting substrates, have been found to impact the catalytic performance of SAzymes. This may consist of the manipulation of the crystal plane, surface coating, elemental composition, heteroatom doping, selection of cofactor mimics, among others.[37] Thus far, a great many SAzymes have been successfully prepared via both bottom-up and top-down synthetic procedures. The former mainly involves atomic layer deposition (ALD),[38, 39] ball milling,[40, 41] wet Chemistry,[42, 43] mass-selected soft landing,[44, 45] and photochemical reduction,[46, 47] while the latter entails high-temperature pyrolysis[48, 49] and gas-phase migration[50, 51]. Below is a summary of these synthetic methods.

Pyrolysis is a widely used method for the synthesis of SAzymes.[52] Experimentally, a metal salt precursor is incorporated into a select organic matrix (typically containing nitrogen). The mixture is then pyrolyzed at elevated temperatures under an inert atmosphere, where the nitrogen atoms are doped into the resulting carbon scaffold and the metal atoms form an M-N-C coordination structure with the carbon and nitrogen atoms (e.g., M = Fe, Cu, Co, Zn, Mn,

etc.).[53] Among these, metal-organic frameworks (MOFs) are the commonly used support materials due to their structural diversity and functional tunability.[54] Under high temperature and inert atmosphere, the organic ligands are carbonized and the metal nodes within the MOFs are converted into metal sites uniformly dispersed within the carbon support. For example, zeolitic imidazole framework-8 (ZIF-8) has been used as a precursor for the preparation of Fe SAzymes consisting of FeN_4 and FeN_5 active sites through a melamine-mediated two-step pyrolysis strategy, where the coating of a SiO_2 shell inhibits nanoparticle aggregation during high-temperature pyrolysis (**Figure 2**).[55]

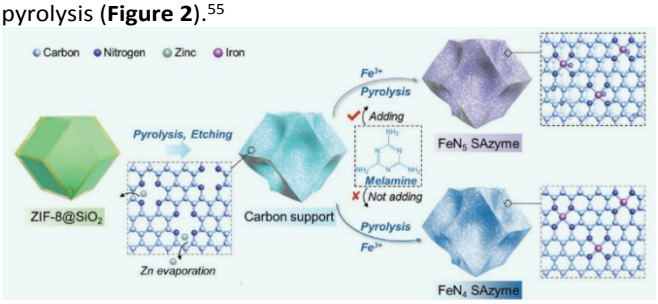


Figure 2. Schematic illustration of the synthesis of FeN_5 SAzyme from a ZIF-8 precursor. Reproduced with permission from ref. 55, copyright 2022, Wiley.

However, pyrolysis at high temperatures usually produces metal (oxide)-containing nanoparticles, resulting in a heterogeneous structure. Note that whereas the nanoparticles may be removed by, for instance, acid etching, the removal is generally incomplete.[56] In addition, during pyrolytic treatment of MOFs, the framework structure may collapse, leading to rearrangement of the carbon skeleton, which may complicate the structure and compromise the accessibility of the M-N-C moiety.

Wet-chemistry strategies have also been employed extensively for SAzyme synthesis.[57, 58] In such procedures, metal-containing precursors are immobilized onto a suitable support via impregnation, co-precipitation, ion exchange or electrostatic absorption, followed by drying and calcination to remove the undesired ligands. In the wet-chemistry procedures, it is important to strengthen the interactions between the metal atoms and the support, and concurrently prevent the aggregation of the metal atoms. This can be achieved with a substrate containing carbon, oxygen and nitrogen, due to strong binding of the metal atoms. This can be further enhanced with structural defects on the supports surface. Indeed, defect engineering can be exploited for the ingenious design of the coordination structures and hence manipulation of the catalytic activity. Notably, the obtained SAzymes can be readily dispersed in aqueous media; yet, a large size may impede the in vivo activity.[35, 59]

ALD is another effective strategy to prepare SAzymes, which is based on a series of continuous self-limiting reactions occurring between the surface of an active precursor substance and an active substrate material.[60] The self-limiting characteristics promote the spontaneous formation of regular geometric structure of SAzymes, a unique feature to study the correlation between the materials structure and catalytic activity.[61]

Other methods, such as atom trapping[62] and gas-phase migration[63], are also emerging. These allow the development of a diverse range of experimental methods for SAzyme preparation.

3. SAzyme Supports

Due to a high surface free energy, metal atoms tend to aggregate into nanoclusters or nanoparticles. It is therefore vital to select an appropriate supporting substrate to stabilize the individual metal atoms and prevent the aggregation. Within this context, two structural scaffolds have been used in the preparation of SAzymes, one carbon-based and the other noncarbon-based.[64]

3.1 Carbon-based supports

Carbon-based supports mostly consist of graphene, graphene oxide, carbon nanotubes (CNTs), MOFs, and graphitic carbon nitride (g-C_3N_4). These supports are usually doped with N atoms, and can thus be used to form MN_x coordination moiety with the metal atoms (M = Fe, Cr, Zn, Mn, Cu, etc.).[65-68] The exposed area of the active sites and the electron-transfer dynamics can be enhanced by the formidable metal-support covalent coordination.[69] For instance, Cheng et al.[70] designed a CNT-supported SAzyme (CNT/FeNC) consisting of FeN_x moieties, by using NaCl crystals as the removable templates (**Figure 3**). The CNT/FeNC SAzyme catalyzed the decomposition of H_2O_2 to hydroxyl radicals (• OH) via the Fenton reaction. Such a POD-like activity was superior to those of Fe_3O_4 nanoparticles and other Fe-based nanozymes reported earlier. In another study, inspired by the actual shape of the heme cofactor of natural HRP, Lee et al.[71] designed and synthesized FeN_4 single-site-embedded graphene (Fe-N-rGO), and observed a high enzymatic activity and selectivity, which was confirmed by density functional theory (DFT) calculations.

g-C_3N_4 is another effective supporting substrate for SAzymes, which exhibit preeminent POD-like activity in the generation of • OH from H_2O_2 decomposition.[72, 73] However, pristine g-C_3N_4 usually show a poor catalytic performance owing to the defects in the shape and internal structure.[74, 75] Thus, a sharp-tip nanoneedle structure has been designed in order to alleviate the impediment of electron transfer by bulk g-C_3N_4 structure.[76] For instance, Fan et al.[77] fabricated a 3D branched g-C_3N_4 artificial enzyme through the ionothermal assay. The unique sharp-edge morphology formed by the modified synthetic process improved the crystallinity of g-C_3N_4, reduced charge-transfer resistance and accelerated H_2O_2 activation. The performance of g-C_3N_4 can also be enhanced by metal or non-metal doping. Jiang et al.[78] prepared g-C_3N_4 composed of sulfur-doped and sulfur-free active sites via liquid sulfur-mediation, where the doping of sulfur atoms led to a significant improvement of the catalytic performance. Likewise, the deposition of noble metal atoms (such as Pt, Pd, and Au) was found to induce localized surface plasmon resonance (SPR) which played a vital role in enhancing the catalytic activity.[79] In fact, defect engineering,[80] modification

tactics,[81] and heterojunctions construction[82] have been employed to improve the catalytic performance of g-C_3N_4.

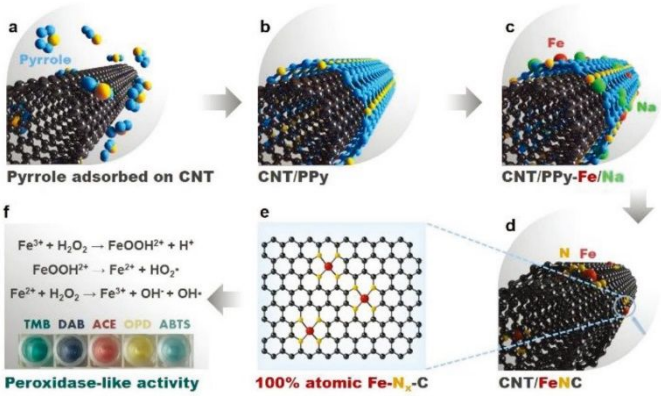


Figure 3. Schematic illustration of the synthesis and catalytic mechanism of Fe SAzymes using carbon nanotubes as a support substrate. (a) Adsorption of pyrrole molecules on CNT through π-π interactions. (b) Addition of ammonium peroxydisulfate induces pyrrole polymerization to obtain polypyrrole-coated CNT (CNT/PPy). (c) Adsorption of metal cations on CNT/PPy from the solution of $Fe(NO_3)_3$ and NaCl. (d) CNT/FeNC SAzyme obtained by pyrolysis of CNT/PPy with adsorbed metal ions in N_2 and then NH_3. (e) Fe-Nx-C moieties in CNT/FeNC SAzyme. (f) POD activity of CNT/FeNC SAzyme in catalyzing the Fenton reaction to generate hydroxyl radicals (·OH). This can be exploited for the oxidative degradation of 3,3′,5,5′-tetramethylbenzidine (TMB, blue), di-azo-aminobenzene (DAB, gray), 3-amino-9-ethylcarbazole (AEC, red), o-phenylene diamine (OPD, yellow), and 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS, green). Reproduced with permission from ref. 70, copyright 2019, Wiley.

MOFs, another important class of carbon-based supports, are coordination polymers, which possess a porous and periodic network structure formed by the self-assembly of transition metal ions and organic ligands, and hence a large specific surface area and tuneable pore size. This enables intimate contact between the substrates and metal atoms and endows MOFs with artificially designed active sites and adjustable catalytic activity.[83, 84] Indeed, MOF-based bioinspired nanomaterials with unique chemical activities, such as photodynamic, sonodynamic, and enzymelike activities, have found diverse biomedical applications, including tumor therapy, antibacterial therapy and antioxidant therapy (**Figure 4**). For instance, four pyrrolic nitrogen sites can form a plane-square structure, which is similar to a porphyrin motif. The center of the four sites acts as a solid trap to bind metal atoms and the surrounding N containing electron lone pairs as donors to coordinate with the target metal atoms.[85, 86] Compared with metal-free porphyrins, metalloporphyrin-based organic linkers containing MN_x sites are formed when the two pyrrole protons (N-H) in the rigid macrocycles of porphyrin are replaced by metal ions, forming MN_x sites, which display enhanced catalytic activity due to the optimized electronic structure and accelerated electron transfer.[87] Therefore, MOFs with different structures and functions can be constructed through self-assembly between metal nodes and porphyrin-based organic linkages, such as tetrakis(4-carboxyphenyl)porphyrin (H_2TCPP), 5,10,15,20-tetrakis(4-aminophenyl) porphyrin (TAPP), 5,10,15,20-tetrakis(4-pyridyl)porphyrin (H_2TPyP), (dicarboxyphenyl)porphyrin (BDCPP), and so on.[88] For example, manganese porphyrin-based MOFs (Mn-MOFs) have been found to exhibit unique SOD- and CAT-like activity, which can remove superoxide anion free radicals, and have been used in tumor immunotherapy and nanovaccine fields.[89]

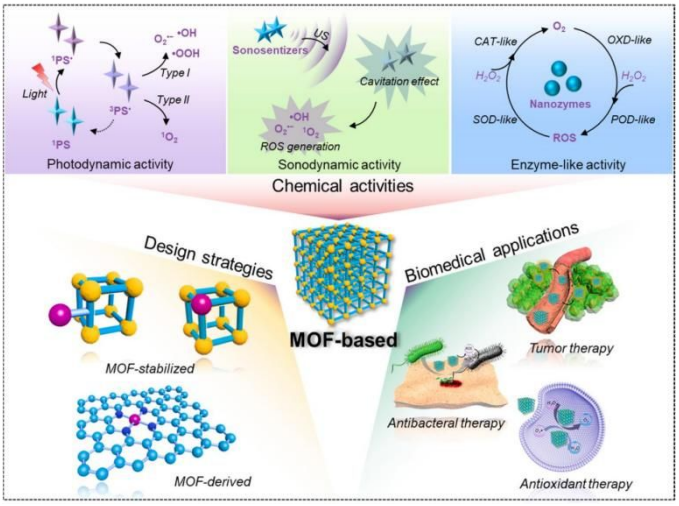


Figure 4. Design strategies and biomedical applications of MOF-based bioinspired nanomaterials with chemical activities. Reproduced with permission from ref. 84, copyright 2023, Elsevier.

Extensive research efforts have also been devoted to covalent organic framework (COFs).[90] The 2D structure of COFs furnishes a high-ordered arrangement and conjugated skeleton, along with a number of hollow sites for stable anchoring of transition-metal centers.[91] For instance, Dong et al.[92] prepared a 2D COF where the periodic organic building blocks were used to stabilize Pt single atoms while the heteroatom-rich (C, N, and O) pore walls afforded unique coordination environments to increase metal atom loading, leading to an increase of the active sites and catalytic activity. In another study,[93] Liu's group prepared a bipyridine-rich COF conjugated with Co single atoms via out-of-plane coordination. Note that in recent years, the COF-derived products are mostly used for photocatalytic applications,[94, 95] with few as biological enzymes.[96] Further research is strongly desired.

3.2 Noncarbon-based supports

Noncarbon-based supports also attract great attention. Among these, transition metal oxide nanomaterials have become one of the most promising scaffolds.[97] The structural defects on the surface of metal oxides can act as binding sites for anchoring single metal atoms. Meanwhile, the distinctive crystal phases and rich electron configurations afford opportunities to form different coordination structures between the metal atoms and scaffolds. Indeed, a great quantity of transition metal oxide-based SAzymes have been prepared. Among these, TiO_2 has been used extensively as a support material, due to strong interactions with the metal atoms. Notably, Qiao's group prepared a Pt-TiO_2 SAC and noticed that metal-support interaction stemmed from the coordination environment rather than physical adsorption or sedimentation.[98] In fact, the metal oxide supports not only immobilize the dispersed metal atoms but also stabilize the geometric structure and modify the electronic structure, which can boost the catalytic performance.[99] Mechanistically, the lattice confinement in the internal structure of the supports acts as substitutable sites for the target metal atoms. In

contrast to the defect-stabilized structure, lattice confined sites are easier to be replaced.[100] Jiang's group synthesized a Pd/MnO_2 SAC with lattice-restricted structures, owing to the spatial constraint of Pt atoms by MnO_2, which spontaneously extracted surrounding lattice oxygen at room temperature with an ultralow energy barrier.[101] The unique coordination environment formed by the metal atoms and metal oxide supports can significantly boost the catalytic activity towards a range of reactions, such as carbon monoxide oxidation reaction and decomposition reaction of hydrogen peroxide.

Transition metal sulfides have also served as effective supports. For instance, cobalt can be atomically dispersed onto a MoS_2 scaffold, and the resultant Co-MoS_2 SAzyme exhibits apparent POD-like activity.[102] In summary, appropriate supports play a critical role in tuning the active sites, improving the catalytic performance and biological applications of SAzymes, which will be highlighted below.

4. Enzymatic Activity of SAzymes

A range of SAzymes have been prepared and used in diverse applications, in particular, biosensing and diagnostics. The metal center is an integral part of the SAzymes, which form M-N-C coordination moieties with surrounding N atoms through metal-N δ bond that mimic the enzyme active centers.[103] Different valence states and electronic structures of the metal atoms give rise to different coordination configuration, and therefore the catalytic activity is closely related to the electron density, size[104] and number[105] of the metal centers. In fact, experimentally, the catalytic activity, selectivity and biological performance can be effectively regulated by manipulation of the metal species, coordination environment, heteroatomic doping and surface modification.[36] Below we will summarize the development of leading SAzymes within the context of the metal centers (Table1).

Table1. Summary of leading SAzymes with different metal centers

Coordination structure	Synthetic method	Characterization	Enzyme-like activity	Biomedical applications	Ref.
Fe-N_4	pyrolysis	TEM, XRD	POD	biosensing	[107]
Fe_{55}-N-C	chemical coprecipitation and pyrolysis	SEM, TEM, HRTEM HAADF-STEM, XPS, XRD	POD	biosensing	[108]
Fe-N-C	wet-chemistry and high temperature calcination	SEM, TEM, HRTEM, STEM, XPS, XRD	POD	antibacterial therapy	[110]
Fe-N_4	pyrolysis and etching	TEM, STEM, XRD, XPS	GOx and HRP	biosensing	[111]
Fe-N_5	Hydrothermal synthesis	TEM, HAADF-STEM	OXD	biosensing and antibacterial therapy	[112]
Co-N-C	wet-chemistry	SEM, TEM, XRD	POD	biosensing	[114]
Co–N–C	hydrothermal and pyrolysis	TEM, HRTEM, STEM, XPS, XRD	OXD	biosensing	[115]
Co–N–C	coordination-pyrolysis-corrosion	TEM, HAADF-STEM, XANES, EXAFS	CAT	cancer therapy	[116]
Zn–N–C	pyrolysis	TEM, HAADF-STEM, XPS, XRD, EXAFS	POD	biosensing and antibacterial therapy	[118]
Pt–N–C	ionothermal method	SEM, TEM, HAADF-STEM, XPS	POD	H_2O_2 detection and antibacterial therapy	[128]
Cu-N-C	wet-chemistry	TEM, XRD, XPS	POD, OXD	antibacterial therapy	[130]
Cu–N_4	electrochemical deposition	TEM, HAADF-STEM, EXAFS, XANES, XRD, XPS	ascorbate peroxidase-like (APX-like)	anti-oxidative damage	[27]
Ru–C_6	in situ one-pot multicomponent self-assembly	SEM, TEM, HAADF-STEM, XRD, XPS, NEXAFS	POD	cancer therapy	[164]
Mn–N-C	etching-adsorption-pyrolysis	TEM, HAADF-STEM, EXAFS, XANES, XAFS, XRD, XPS	CAT, OXD, POD	cancer therapy	[171]
Pd–N-C	"top-down" strategy	TEM, HRTEM, HAADF-STEM, EXAFS, XANES, XRD, XPS	POD, GSHOx-mimic	cancer therapy	[165]

Note: TEM, transmission electron microscopy; HRTEM, high-resolution transmission electron microscopy; HAADF-STEM, high-angle annular dark field-scanning transmission electron microscopy; SEM, scanning electron microscopy; XRD, X-ray diffraction; XPS, X-ray photoelectron spectroscopy; EXAFS, extended X-ray absorption fine structure; XANES, X-ray absorption near-edge spectroscopy.

4.1 Fe SAzymes

Fe-centered SAzymes, one of the most promising mimetic enzymes, have been extensively studied in recent decades, where the Fe-N-C coordination moiety serves as the active center due to geometric and electronic effects.[106] Among the numerous strategies of synthesis, controlled pyrolysis of zeolitic-imidazolate frameworks (e.g., ZIF-8) have been the leading method in recent years for the preparation of Fe SAzymes. For instance, Lin's group[107] prepared a FeN_4 SAzyme by pyrolysis of ZIF-8 and observed an unprecedented POD-like activity. Motivated by the structure of hemoglobin, Liu et al.[108] designed a precursor including hemin and ZIF-8 (Hemin@ZIF-8). During pyrolysis hemin served as the doping agent and effectively suppressed the aggregation of Fe atoms. Meanwhile, the larger Hemin would burst the cage of ZIF-8 and broke the confinement effect, creating a microporous multilayer structure and a high surface area. Experimentally, the group synthesized a series of Fe,N co-doped porous carbon, and observed that the POD-like activity in the TMB oxidation reaction varied with the initial feed of hemin, among which an optimal loading was identified.[109] Another noteworthy example is spherical mesoporous Fe-N-C SAzyme, which featured a large pore size (4.0 nm), high specific surface area (413.9 m^2 g^{-1}), uniform diameter (100 nm) and highly dispersed iron atoms.[110] These characteristics facilitated mass transport and accessibility of the active sites, and hence boosted the POD-like activity.

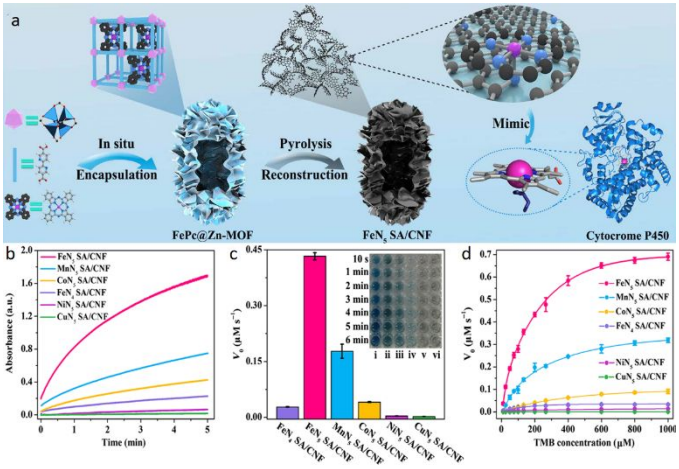


Figure 5. (a) Schematic formation process of carbon nanoframe–confined atomically dispersed Fe sites with axial N coordination for mimicking the active center of cytocrome P_{450}. (b) Comparison of the OXD-like activity of a series of nanozymes with a similar MN_5 structure (M = Co, Mn, Ni, and Cu), as manifested by the time-dependent absorbance of oxidized TMB (oxTMB) at 652 nm. (c) Histogram of the initial reaction rate (V_0) and (d) typical Michaelis-Menten curves in the presence of (i) FeN_5 SA/CNF, (ii) MnN_5 SA/CNF, (iii) CoN_5 SA/CNF, (iv) FeN_4 SA/CNF, (v) NiN_5 SA/CNF, and (vi) CuN_5 SA/CNF in air-saturated sodium acetate–acetic acid buffer. The inset to (c) is the photographs of the TMB solution in the presence of the various catalysts for up to 6 min. Reproduced with permission from ref. 112, copyright 2019, AAAS.

In a recent study,[111] we developed a platform for glucose sensing based on Fe SAzyme with a dual-signal readout mode based on fluorescence and electrochemistry. Experimentally, 3D porous N-doped carbon aerogels were used as the support matrix in which Fe single atoms were embedded to achieve uniform dispersion and maximize atomic utilization. Specifically, with SiO_2 nanoparticles as the structural templates, a gelatin–zinc hydrogel containing iron(II) phenanthroline ($Fe(PM)_3^{2+}$) was exploited as the precursor and subject to repeated freeze-drying at -20 °C. The resulting biomass hydrogel was then transformed into N-doped carbon aerogel anchored with Fe single atoms (NCAG/Fe) by pyrolysis and acid etching, which possessed a large surface area and rich mass transport channels and exhibited outstanding POD-like properties due to the formation of FeN_4 moieties. In fact, nonfluorescent OPD could be oxidized to fluorescent 2,3-diaminophenazine (DAP) by H_2O_2 generated by NCAG/Fe-catalyzed oxidation of glucose, and the fluorescence signal intensity varied with the glucose concentration; concurrently the oxidation of glucose also generated a significant electrochemical signal. Such a dual-signal detection platform could be exploited for the accurate and reliable detection of glucose even in clinical serum samples and artificial body fluids, as compared to commercial sensors.

Notably, Fe SAzymes may consist of not only FeN_4 but also FeN_5 coordination moieties, and the latter show a better catalytic activity, owing to the optimized coordination structure. For instance, inspired by the axial ligand-coordinated heme of cytochrome P_{450}, Huang et al.[112] rationally introduced a fifth nitrogen atom into the structure of FeN_4 to form axial coordination with the central iron atom. In the new assay, the organic nitrogen linkers were converted to pyridinic nitrogen at high temperature, which coordinated with the FeN_4 sites that were separated under the constraint of the carbon nanoframes (**Figure 5a**). The axial-coordination N atom of FeN_5 effectively activated O_2 and facilitated the cleavage of the O-O bond, behaving like natural oxide enzymes. At the same time, they systematically studied a series of nanozymes with a similar MN_5 structure (M = Co, Mn, Ni, and Cu), from which FeN_5 SA/CNF was found to exhibit the highest OXD-like activity, stability and biocompatibility (**Figure 5b-d**). Results from DFT calculations were highly consistent with the experimental conclusion. Taken together, these results show that the emergence of FeN_5 SAzymes provides a valuable reference in uncovering and understanding the enzyme-like mechanism and paves the way for ingenious formulation and appropriate applications of SAzymes.

4.2 Co SAzymes

As the heteromorphism of Fe_3O_4, the enzyme-like properties of Co_3O_4 nanoparticles have attracted wide attention, where the catalytic activity can be improved by changing the shape and increasing the specific surface area.[113] For instance, Wang's group[102] used the single-atom Co-MoS_2 nanozymes as a model, and combined experimental and theoretical studies to show that the Co centers and the MoS_2 supports involved different catalytic mechanisms: the former entailed an electron-transfer mechanism, while the latter was based on a Fenton-like reaction. The synergistic interactions between the Co single atoms and MoS_2 substrates greatly improved the POD-like activity of Co-MoS_2, which was successfully applied in the colorimetric and electrochemical sensing of hydrogen peroxide.

In another study,[114] Co SAzymes were pyrolytically derived from ZIF-67 and a Cobalt salt template featuring unsaturated Co-porphyrin centers, and the resultant Co-PMCS SAzymes

exhibited excellent POD-like and other enzyme-like activities. This was then exploited for the colorimetric detection of a series of antioxidants by taking advantage of the color change of TMB oxidation by H_2O_2 catalyzed by the Co-PMCS SAzyme, where the color diminished in the presence of varied antioxidants. The sensor assay was demonstrated with 7 antioxidants, and exhibited a low detection limit, high accuracy, and good anti-interference capability. Sun's group exploited the inhibition of the OXD-like activity of Co-N-C SAzymes by thiols for the detection of biothiols (e.g., glutathione (GSH), cysteine (Cys)),[115] as biothiols could bind to the Co centers, inhibit the adsorption of H_2O_2, and thus block the oxidation process. Chen's group prepared a Co-SAs@NC SAzyme via a coordination-pyrolysis-corrosion process and observed CAT-like activity.[116] In the tumor microenvironment, the SAzyme first acted as a CAT to decompose the endogenous H_2O_2 of tumor cells to O_2, and then functioned as an OXD to catalyze the conversion of O_2 into highly cytotoxic $O_2^{\bullet-}$ radicals which led to apoptosis of the tumor cells.

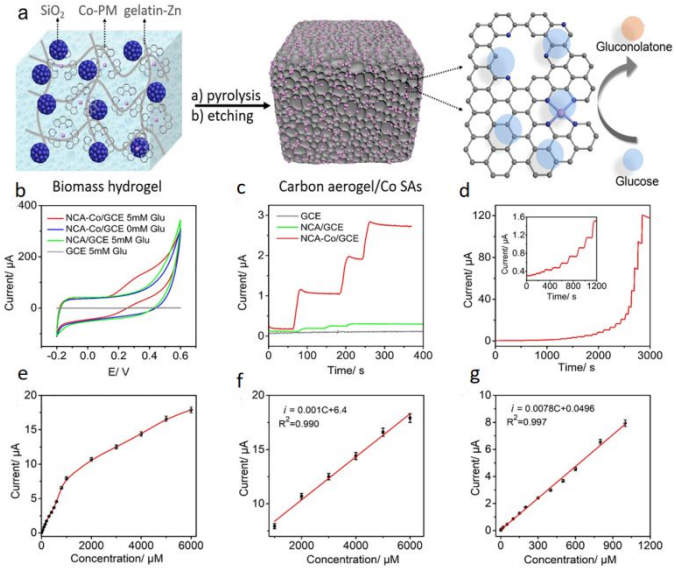


Figure 6. (a) Schematic of the preparation of NCA-Co aerogels derived from biomass hydrogels. (b) Cyclic voltammograms in 0.1 M NaOH at the scan rate of 50 mV s^{-1} of NCA-Co/GCE (GCE, glassy carbon electrode) in the absence and presence of 5 mM glucose, NCA/GCE and bare GCE in the presence of 5 mM glucose. (c) Chronoamperometric profiles of different modified electrodes at the applied potential of +0.3 V with the successive additions of 100 μM glucose into 0.1 M NaOH. (d) Chronoamperometric curve of the NCA-Co/GCE electrode upon the successive addition of glucose at various concentrations (0.5, 1, 2, 10, 20, 50, 100, 150, 200, 300, 400, 500, 600, 800, 1000, 2000, 3000, 4000, 5000, 6000 μM) into a 0.1 M NaOH solution at the applied potential of +0.3 V. Inset is the magnified segment for the glucose concentration range of 0.5 to 100 μM. (e) Current signals at different glucose concentrations and the corresponding linear correlation between the oxidation current increment and glucose concentration in the range of (f) 0.5–1000 μM and (g) 1000–6000 μM. Reproduced with permission from ref. 117, copyright 2022, Elsevier.

In a recent study,[117] following the previous procedure of the preparation of NCAG/Fe, we synthesized a Co SAzyme based on the similarly structured N-doped carbon aerogels. Again, the gelatin-zinc hydrogel was adopted as the precursor, into which were added a Co-PM complex and SiO_2 nanoparticles. The hydrogel was then pyrolytically converted into honeycomb carbon aerogels embedded with Co single atoms (NCA-Co) consisting of three active sites, CoN_4 in the basal plane, as well as CoN_4 and CoN_3 at the edge of the aerogel nanopores (**Figure 6a**). Theoretical calculations showed that the adsorption energy of glucose at the different metal sites varied in the order of basal CoN_4 (−1.047 eV) < nanopore-edge CoN_3 (−0.811 eV) < nanopore-edge CoN_4 (−0.634 eV), suggesting the high catalytic activity of cobalt sites at the nanopore edges. When the NCA-Co nanozyme was used for electrochemical detection of glucose, good linearity was observed between the glucose concentration and current signal, due to the POD-like activity of the NCA-Co SAzyme (**Figure 6b-g**).

4.3 Zn SAzymes

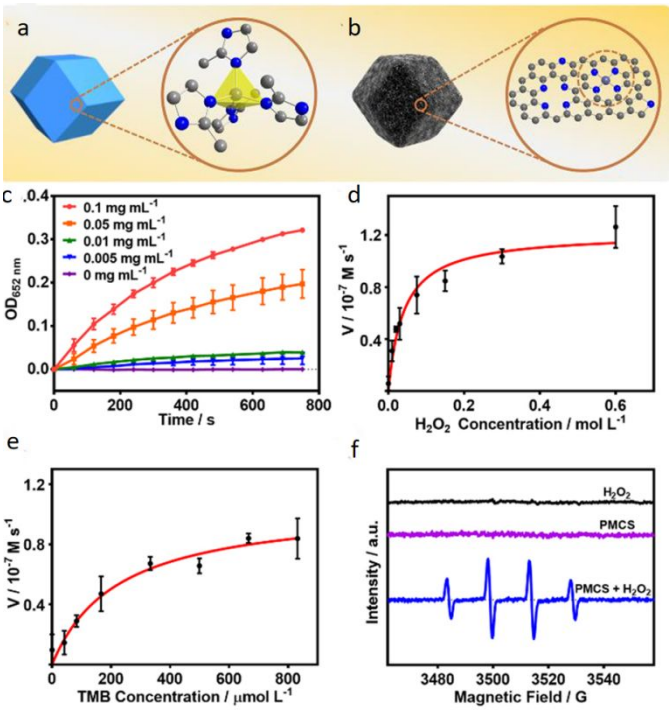


Figure 7. (a) Schematic illustrations of ZIF-8 with the ZnN_4 tetrahedral motif and (b) PMCS with the porphyrin-like structural model. (c) POD-like activity of PMCS at different concentrations in the catalytic oxidation of TMB. Steady-state kinetic assay of PMCS for (d) H_2O_2 and (e) TMB. (f) ESR spectra demonstrating •OH generation by H_2O_2, PMCS, and PMCS + H_2O_2. Reproduced with permission from ref. 118, copyright 2019, Wiley.

ZIFs are a kind of MOFs with a zeolite-like framework structure, which are produced by the reaction of metal ions, such as divalent Zn and Co, and imidazole or their derivative ligands in organic solvents, among which zinc-based ZIF-8 is the most commonly used ZIF and can be used to derive carbon nanocomposites containing atomically dispersed zinc atoms (**Figure 7a**).[118] With the addition of target metal ions of a similar size of Zn^{2+} (e.g., Co^{2+}, Ni^{2+}, Fe^{2+}, etc.), the target metal ions can be homogeneously distributed into the metal nodes of the ZIF framework to form bimetallic ZIFs through coordination with the 2-methylimidazole linker. As the Zn species become evaporated at high temperatures, porous carbon is formed and embedded with the target metal ions. For ZIF-8 derived carbon (**Figure 7b**), the Zn-centered porphyrin-like moiety endows the sample (denoted as PMCS) with a POD-like activity, as manifested in the catalytic oxidation of TMB in the presence of H_2O_2 (**Figure 7c-e**). The unsaturated ZnN_4 coordination configuration acts as active sites and can catalyze the decomposition of H_2O_2 into hydroxyl radicals (•OH), as confirmed in electron spin resonance (ESR)

measurements (**Figure 7f**). The optimal reaction conditions are similar to those for HRP, and the high activity can be retained within a wide range of pH and temperature. Xu et al.[119] synthesized Mo/Zn dual-atom nanozymes supported on a macroscopic amphiphilic aerogel by pyrolysis of poly(vinyl alcohol) (PVA) and supramolecular coordination complexes.[120] The macroscopic dimension of the PVA substrate not only greatly enhanced the metal atom loading, but also stabilized the metal atoms. The Zn/Mo dual SAzyme showed a high metal content (1.5 and 7.3 wt% of Zn and Mo, respectively) without acid etching and with no structural collapse of the support, and synergistically led to a POD-like activity.

Of the Zn-based nanocomposites, the ZnN_4 moieties have been known to exhibit a high catalytic activity since the N atoms coordinating to the Zn center significantly lower the energy barrier and accelerate the electron-transfer kinetics. In fact, Zn SAzymes have shown potent antibacterial activity without external stimulation and toxicity to tissues and organs, in contrast to their nanoparticle forms.[121, 122]

4.4 Pt SAzymes

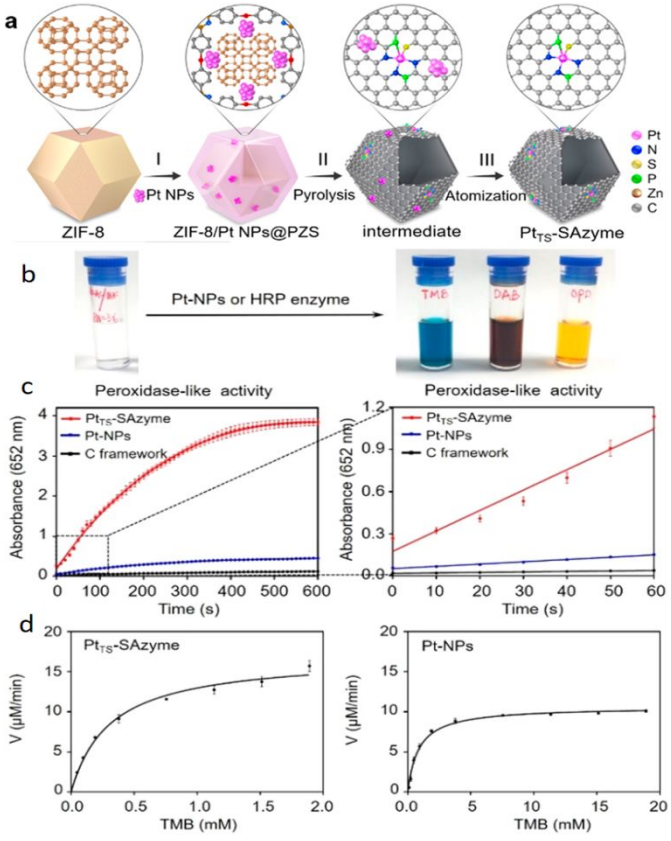


Figure 8. (a) Illustration of the preparation process of Pt_{TS}-SAzyme. (b) POD-like activity of Pt-based catalyzing the oxidation of POD substrates of TMB, DAB, and OPD to produce a color change. (c) (Left) Reaction–time curves of TMB oxidation catalyzed by PtTS-SAzyme, Pt-NPs nanozyme, or Pt-free inorganic C framework (NPS-HC); (Right) zoom in of the initial linear portion of the reaction–time curves. (d) Reaction kinetics of (Left) Pt_{TS}-SAzyme and (Right) Pt-NPs nanozyme. Reproduced with permission from ref. 127, copyright 2021, American Chemical Society.

Since the first report from Zhang' group that Pt/FeO_x SACs exhibited a high atom utilization and unexpected catalytic activity, Pt SAzymes have attracted widespread attention.[123] A host of Pt SACs have been produced and widely used in a range of applications,[124] with CeO_2 being a unique support material.[125] For instance, Yan et al.[126] used Pt-CeO_2 SAzymes for the production of a new type of bandage for brain trauma. In vitro and in vivo experiments disclosed that the nanozyme-based bandage afforded sustained multienzyme activity (including POD-, CAT-, SOD-, and GPx-like activities), nontoxicity and excellent durability. The bandage significantly improved wound healing and reduced inflammation, which set a precedent for the noninvasive therapy of SAzymes.

Liang et al. developed a practical strategy for engineering high-performance nanozymes by reversing the thermal sintering process, which atomized platinum nanoparticles into single atoms within a carbon matrix derived from ZIF-8 (**Figure 8a**).[127] The P and S dopants not only promoted the atomization of Pt nanoparticles to Pt_{TS}-SAzyme, but also facilitated the formation of Pt_1N_3PS active moieties due to electron donation of the P dopants and electron acceptance of the N and S dopants. The resultant Pt_{TS} SAzyme was found to exhibit a remarkable POD-like activity by catalyzing the oxidation of POD substrates (e.g., TMB, DAB, and OPD) to produce a color change (**Figure 8b**). After thermal atomization of Pt nanoparticles into Pt single atoms, the POD-like catalytic activity and initial reaction velocity of Pt_{TS}-SAzyme were significantly higher than those of Pt nanoparticles or Pt-free N, P and S co-doped hollow carbon polyhedron (NPS-HC) (**Figure 8c-d**). Pt SAzymes have also been prepared with Pt single atoms supported on g-C_3N_4 via a wet chemistry strategy with alkali metal ions-intercalated g-C_3N_4 (g-C_3N_4-K) and a platinum salt precursor. The resulting SA-Pt/g-C_3N_4-K SAzyme remarkably enhanced the generation of •OH radicals by reducing the desorption energy of the intermediate state of OH* from the active site during H_2O_2 activation.[128] This opens up a new way in the antibacterial application of Pt SAzymes.

4.5 Other SAzymes

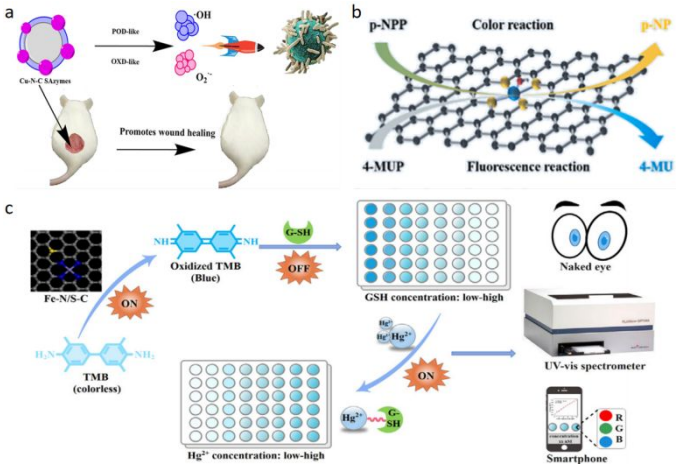


Figure 9. (a) Schematic diagram of the antibacterial activity of Cu-N-C SAzymes with intrinsic POD- and OXD-like activity. Reproduced with permission from ref. 130, copyright 2022, American Chemical Society. (b) Schematic diagram of the catalytic mechanism of Ce–N–C SAzyme with PPA-like activity for the rapid and sensitive detection of aluminum ions through color change and fluorescence signal generation during the catalytic process. Reproduced with permission from ref. 131, copyright 2022, Elsevier. (c) Schematic diagram of a colorimetric sensor based on Fe-N/S-C SAzymes for simultaneous multimode detection of GSH and Hg^{2+}. Reproduced with permission from ref. 137, copyright 2022, Elsevier.

Other metals have also been used to prepare SAzymes. Among the metals with bactericidal effect, copper can effectively inhibit the growth of various harmful bacteria, viruses and microorganisms in water (such as algae). However, as a heavy metal, the biological applications of copper and its compounds have remained limited due to metal toxicity.[129] The metal leaching effects can be minimized with Cu SAzymes. Zhu et al. observed that Cu SAzymes with Cu-N-C moieties exhibited intrinsic POD- and OXD-like activity to catalyze the generation of •OH and $O_2^{\bullet-}$ from H_2O_2 and O_2, respectively.[130] The produced ROS displayed a significant activity towards the inhibition of bacterial growth. As the antibacterial activity of the Cu SAzymes was significantly superior to vancomycin, a common antibacterial drug (**Figure 9a**), it is anticipated that Cu SAzymes will play a critical role in the development of new antibacterial agents and new antimicrobial strategies.

Song et al. observed an excellent phosphatase-like (PPA-like) activity with Ce-N-C SAzymes, which effectively catalyzed the dephosphorylation process of inorganic phosphate.[131] Notably, as Al^{3+} could form Al-O bond via specific binding with O atom and inhibit the PPA-like activity (**Figure 9b**), a fast, low cost, portable, and efficient fluorescent liquid-phase sensor can be constructed for the sensitive detection of Al^{3+}.

Some other metals, such as Ru, Co, Au, and Se, have also been used as the active centers for SAzyme.[132] They are usually synthesized by wet chemistry and high-temperature pyrolysis. Notably, two different metal atoms can be combined to construct dual-atom structures, where the synergistic effects can be exploited for the manipulation of the electronic structure of the metal centers.[133] In earlier studies, these materials were mainly used as electrode catalysts for electrochemical energy technologies. In recent years, they have shown great potential in biomedical applications, such as tumor therapy and biosensing, with the continuous optimization of the structures and emergence of a variety of enzyme-like activities,[134] as demonstrated below in section 4.6.

4.6 Nonmetal dopants

For SAzymes, the M-N-C active moiety can be further engineered with the introduction of additional heteroatom dopants, such as O, S, and P, by taking advantage of their different electronegativity to manipulate the electronic property of the metal centers.[135] For instance, in comparison to the FeN_4 SAzyme and Fe_3O_4 nanozyme, FeN_3P SAzyme by P doping into the Fe-N-C SAC exhibited markedly enhanced POD-like activity, as manifested in the colorimetric reaction of hydrogen peroxide and TMB.[136] DFT calculations showed that the adsorption energy of hydrogen peroxide was lowered to -0.40 eV on FeN_3P, as compared to –1.24 and -0.60 eV on $Fe_3O_4(111)$ and FeN_4, respectively, consistent with the enhanced kinetics of the dissociation of hydrogen peroxide into two surface OH species ($H_2O_2 \rightarrow 2OH$). Subsequently, in the FeN_3P SAzyme, FeN_4 SAzyme and $Fe_3O_4(111)$ nanozyme, the two surface OH species reacted with each other to form surface O and H_2O molecule ($2OH \rightarrow O + H_2O$) with an energy barrier of 0.49, 1.05 and 1.51 eV, respectively, which indicated that this process was most easily carried out on the surface of FeN_3P SAzyme. However, since the H_2O molecules were still adsorbed on the surface of the nanozyme, this could inhibit the catalytic process. A thermodynamic investigation was then conducted to examine the desorption of water molecules. The results showed that the desorption energy of water molecules was relatively low on the surface of all the three nanozymes under neutral conditions. In acidic media, the reaction potential energy of the transformation of OH species into H_2O on FeN_4 SAzyme was increased to 2.84 eV, suggesting that OH would be stuck to the Fe centers and impede the catalytic process; by contrast, OH could be instantaneously converted into H_2O on $Fe_3O_4(111)$, due to an ultralow reaction potential energy of 0.11 eV, which also led to failure of the catalytic process. Yet, the reaction potential energy of this process on FeN_3P SAzyme was almost independent of solution pH, indicating that the surface OH species could be successfully converted into H_2O. The desorption of H_2O can also occur easily due to a low barrier on the three nanozymes. In summary, the proposed reaction pathway demonstrated an enhanced catalytic activity of FeN_3P SAzyme due to synergistic effects of the P dopants through the precise coordination to the metal centers. Similarly, Li et al.[137] introduced S dopants into Fe-N-C SAzyme, which not only manipulated the geometric configuration and electronic structure of the metal centers, but also reduced the formation of inactive Fe carbide, and facilitated the formation of a porous carbon structure with a high specific surface area. The resulting Fe-N/S-C SAzyme exhibited enhanced OXD-mimicking activity due to the auxiliary regulation of the S atoms, as compared to the Fe-N-C counterparts (**Figure 9c**).

Doping of polynary metals has also been employed to construct SAzymes with a high density of activity sites, where the addition of multiple metals can form active sites and regulate the electronic structure through the interactions between the metals centers.[138] Therefore, the binding energy of reactants, intermediates, and products can be modulated for an optimal catalytic performance. In a recent study,[139] a Fe-Bi bimetallic SAzyme (Fe/Bi-NC) was prepared by using Fe-doped Bi-MOF as the precursor, and consisted of $Fe\text{-}N_4$ and $Bi\text{-}N_4$ dual-sites. The role of Fe was not only to generate active sites, but also to expand the distance of neighboring Bi atoms and prevent their aggregation. Meanwhile, PVP surfactants were added to prevent the interlayer accumulation of the MOF precursor through chemical adsorption. During pyrolysis the precursor was transformed into thin-sheet Fe/Bi-NC, a unique structure conducive to the formation of single-atom sites. Experimental studies and DFT calculations confirmed the positive synergistic effect between the FeN_4 and BiN_4 sites, which markedly enhanced the OXD-like activity. Yet, an Fe content over 4% actually diminished the enzymatic activity. Therefore, accurate regulation of the contents of constituent atoms is critical for attaining ideal bimetallic SAzymes. In another study, Au-Ni/g-C_3N_4 SAzymes were produced with g-C_3N_4 nanosheets doped with Au-Ni bimetallic nanoparticles,[140] which exhibited apparent POD-like activity and could be used for the colorimetric detection of glucose. In enzyme kinetics measurements, the K_m values were found to be lower with the

bimetal SAzymes than those of their monometallic counterparts, indicating enhanced affinity of the Au-Ni/g-C_3N_4 nanocomposite toward the substrates. In fact, the enhanced catalytic activity was attributed to the synergistic effects between the Au and Ni atoms. Due to a clear difference of the ionization potential between Au and Ni (9.22 eV and 7.63 eV), electron transfer occurred from Ni to Au, resulting in an increase in the charge density of Au, such that both Au and Ni could act as active sites for the POD-like activity. This bimetallic synergistic effect has also been previously reported in the literature.[141]

In summary, one can see that metal centers are the active sites that dictate the catalytic activity of SAzymes, due to the formation of unique M-N-C coordination structures.[103] For instance, among the Fe-based SAzymes, the FeN_3 moiety is known to exhibit a strong OXD-like catalytic activity because O_2 molecules adsorb readily onto the FeN_3 centers via a side-on configuration, which is the first step in the OXD-like reaction and plays an important role in the subsequent steps.[142] FeN_4 SAzymes, however, usually exhibit excellent POD-like activity, because the FeN_4 active sites mimics the porphyrin iron cofactor structures (Fe atoms coordinated to four nitrogen atoms in the plane of the porphyrin ring) of natural HRP.[36] For FeN_5 SAzymes, the electronic structure of the Fe centers is optimized, due to the addition of the axial ligand through the nitrogen atom, which facilitates the electron-transfer kinetics and leads to a better OXD- and POD-like activity than that of FeN_3 and FeN_4.[55]

Similarly, with a zinc-centered porphyrin-like structure, Zn-based SAzymes also display outstanding POD-like activity. The unsaturated-coordination ZnN_4 centers can catalyze the decomposition of H_2O_2 into •OH, which is similar to the catalytic pathway of HRP.[143] Cu-based SAzymes not only exhibit OXD- and POD-like activity but also mimic polyphenol oxidase (e.g., tyrosinase, catechol oxidase, and laccase), where the active sites all feature coupled multinuclear Cu(II) centers and can oxidize important biological substrates (e.g., polyphenols and polyamines) and directly activate oxygen.[144]

Notably, these enzymatic activities are sensitively dependent on solution pH. Under different pH conditions with different metal centers, H_2O_2 can be decomposed into different forms. Specifically, SAzymes typically exhibit POD-like activity at low pH and CAT-like activity at high pH.[145] The number of coordinating nitrogen can also impact the catalytic activity. For example, when the number (*x*) of coordinating N in CoN_x decreases from 4 to 2, Co SAzymes show an enhanced activity towards oxygen reduction reaction,[146] while FeN_5 SAzymes display a better OXD- and POD-like activity than both FeN_4 and FeN_3.

Therefore, to improve and optimize the catalytic performance of SAzymes, the elemental composition, metal coordination configuration, metal loading and supports interactions need to be systematically varied and carefully examined,[104] as they affect the geometric configuration and electronic structure of the metal centers, resulting in different catalytic activity and selectivity. This is of particular importance for SAzymes that possess multi-enzyme activity and find diverse applications in antibacterial, cancer therapy, and cell protection.[147]

5. Biological and Medical Applications

5.1 Biosensing

Due to the low cost, high efficiency, high sensitivity and great stability, SAzymes have been successfully utilized in the construction of a variety of biosensors based on colorimetric, electrochemical and fluorescence platforms.[148] Most colorimetric biosensors are based on the TMB redox chemistry, as TMB can be oxidized to a blue product (oxTMB) catalyzed by the POD-like activity of SAzymes, which can be readily detected with an absorption peak at 652 nm, and the concentration of the target analyte can be determined from the absorbance.[149] For instance, Zhou et al.[150] fabricated an Fe SAzyme by an isolation-pyrolysis procedure which displayed POD-like activity and catalyzed the oxidation of TMB in the presence of H_2O_2 generated from the oxidation of galactose with galactose oxidase (Gal Ox) (**Figure 10a**). The colorimetric sensor exhibited a good linear relationship between the optical absorbance and galactose concentration, with a good minimum detection limit, which paved the way for a rapid and economical detection of galactose and diagnosis of galactosemia. Similarly, in a Bi SAzyme anchored onto Au hydrogels (BiSA@Au),[151] the Bi atoms and the unique porous nanowire networks of the hydrogels greatly improved the catalytic activity by decreasing the potential barriers for the decomposition of H_2O_2. With the immobilization of GOx on the surface of the BiSA@Au nanozyme, the oxidation of TMB could be exploited for the accurate and sensitive colorimetric detection of glucose in clinical samples. In another study with FeN_3/PtN_4 dual-atom SAzymes,[152] a colorimetric sensing platform was obtained for the quantization of dopamine whereby the POD-like activity of FeN_3/PtN_4 was utilized to catalyze the oxidation of TMB by H_2O_2 to generate blue oxTMB, and then the addition of dopamine reduced oxTMB back to colorless TMB (**Figure 10b**). Similarly, Feng et al. synthesized B-doped Zn-N-C (ZnBNC) SAzymes for the colorimetric detection of p-phenylenediamine (PPD), a reductive carcinogen,[153] which reduced blue oxTMB to colorless TMB, with a linear decrease of the absorbance with PPD concentration (**Figure 10c**).

Notably, the oxidation of TMB can be inhibited by a range of small-molecule compounds that are effective indicators of human health, such as uric acid, dopamine, norepinephrine and ascorbic acid. This unique chemistry can be exploited for the development of biosensors for their indirect detection.[114] For instance, Wu et al. prepared an Fe SAzyme with a high OXD-like activity at pH 3.0, and the activity could be effectively inhibited by 4-acetamidophenol (AMP) via a reversible mixed-inhibition mechanism.[154] This unique property could be exploited for the detection of AMP.

In electrochemical biosensing, Hu et al.[155] prepared an A-Co-NG SAzyme with Co atomically dispersed into N-doped graphene for electrochemical detection of uric acid by

measuring the change of the current signals involved in the oxidization of uric acid molecules. Liu et al.[156] optimized the Co-N-C structure of a Co SAzyme by embedding Co atoms into a reduced graphene oxide aerogel (rGA) forming a three-dimensional layered electrochemical electrode, which exhibited a high electrocatalytic performance in the in-situ detection of H_2O_2, uric acid and dopamine. By monitoring the current response of Co-N-C, rGA and Co-N-C/rGA on a GCE under different experimental parameters, it was found that Co-N-C/rGA exhibited the best catalytic performance among the series (**Figure 10d**).

Notably, POD-mimicking nanozymes require an acidic medium to exhibit the enzymatic activity, while near-neutral pH is the optimal condition for GOx. The cumbersome steps of switching pH during the sensing operation bring tremendous obstacles to the cascade reaction of the two enzymes. To mitigate these issues, a foldable paper microfluidic device has been rationally designed which is divided into two parts at acidic and neutral pH for the Fe SAzymes and GOx, respectively (**Figure 10e(i)**).[157] Experimentally, glucose is oxidized by GOx under neutral pH generating H_2O_2, which then migrates to the other compartment by folding the paper device producing a colorimetric signal under acidic pH (**Figure 10e(ii)**). The unique technology breaks the pH restriction of cascade reactions and may serve as an innovative portable integrated microdevice for rapid detection of glucose, a key step in the further development of point-of-care testing (POCT) (**Figure 10e(iii)**). In fact, a range of biosensors are being developed within the context of miniaturization, integration and wearability. The devices are portable, exhibit a fast detection speed, and can be integrated with artificial intelligence and informational technology for remote, real-time monitoring. Nevertheless, there are not many examples of SAzymes used in these applications yet. The construction of stable point-of-care testing (POCT) devices with SAzymes instead of natural enzymes is anticipated to greatly extend the sensor lifetime. Improving the stretchability of SAzymes through rational design to build wearable biosensing devices will also create an emerging pathway.[158]

Furthermore, fluorescence-based biosensors have also been fabricated with SAzyemes.[159] For instance, Wang et al.[160] utilized the POD-like activity of Fe SAzymes to catalyze the oxidation of nonfluorescent OPD to fluorescent DAP, with a maximum fluorescence emission peak at 566 nm. The generated DAP efficiently quenched the fluorescence emission of polyvinylpyrrolidone-protected copper nanoclusters (PVP-CuNCs) at 438 nm; yet this could be blocked by thiocholine derived from acetylthiocholine by acetylcholinesterase (AChE). Therefore, the ratio of the fluorescence emission at 566 and 438 nm (F_{566}/F_{438}) could be exploited for the quantification of the AChE activity (**Figure 10f**).

In addition to the above-mentioned biosensors, nanozyme-based immunosensors have also been reported. In a typical sandwich-type immunosensor, the target (antigen) first binds to the corresponding specific antibody, followed by the neuraminidase (NANase)-linked antibody, which then binds to the recognized antigen, thus forming a sandwich structure. For SAzyme-based immunosensors, the optimized electronic structure can lead to a high catalytic activity. The introduction of SAzymes catalyzes some chromogenic reactions, which to some extent compensates for the lack of sensitivity of colorimetric analysis, while maintaining a good activity after prolonged storage within a wide range of pH and temperature.[161]

Furthermore, the accurate detection of DNA usually requires a combination of signal amplification strategies by, for instance, nanozymes. However, the use of SAzymes for this purpose has been relatively rare. It can be envisaged that when a DNA probe is modified with SAzymes that can catalyze the chromogenic reaction of the relevant chromogenic substrate, precise detection of DNA can be achieved in conjunction with a signal amplification strategy.[162]

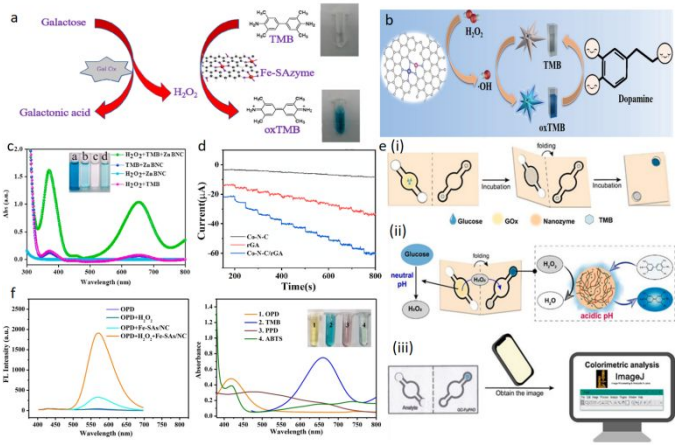


Figure 10. (a) Schematic illustration of the colorimetric assay for galactose detection by an Fe SAzyme. Reproduced with permission from ref. 150, copyright 2020, Elsevier. (b) Schematic illustration of dopamine determination by FeN_3/PtN_4 dua-site SAzymes Reproduced with permission from ref. 152, copyright 2022, Springer. (c) UV–vis spectra of ZnBNC, ZnNC and H_3BO_3–ZnNC in the H_2O_2/TMB solution. Inset is the photograph of the various solution. Reproduced with permission from ref. 153, copyright 2020, Elsevier. (d) Amperometric response of Co–N–C, rGA and Co–N–C/rGA on a GCE with the addition of 100 μM H_2O_2 in 0.01 M PBS (pH 7.4). Reproduced with permission from ref. 156, copyright 20203, Elsevier. (e) Schematic illustration of the colorimetric detection of glucose via PGA-Fe/CS NPs-incorporated foldable paper microfluidic device (PGA-Fe/CS@FμPAD). (i) Glucose detection procedure, (ii) cascade reaction incorporating glucose oxidation under neutral pH and H_2O_2 reduction with TMB oxidation under acidic pH, and (iii) on-site quantification via an image acquired with a smartphone. Reproduced with permission from ref. 157, copyright 2023, Elsevier. (f) (Left) Fluorescence emission spectra of OPD, OPD/H_2O_2, OPD/Fe-SAs/NC, and OPD/H_2O_2/Fe-SAs/NC, and (Right) UV-vis absorption spectra of H_2O_2/Fe-SAs/NC with the substrates of OPD, TMB, PPD and ABTS. Inset is the photograph of the varied solutions. Reproduced with permission from ref. 159, copyright 2020, Elsevier.

5.2 Cancer diagnosis and therapy

SAzymes featuring various enzyme-like activities have also been creatively introduced to assist the diagnosis and treatment of cancers, which is efficient, non-toxic, biocompatible, and non-invasive (**Figure 11**).[163, 164] Ferroptosis is a cell death mechanism discovered in 2012. It is caused by the accumulation of iron-dependent lipid peroxidation (LPO), which induces lethal disruption of the cell membrane structure. Glutathione peroxidase 4 (GPx-4) can reduce the production of LPO by taking GSH as a cofactor, but glutathione oxidase (GSHOx) will promote the conversion of GSH to glutathione disulfide (GSSG), resulting in the deactivation of GPx-4 and thus increasing the accumulation of LPO. In 2021, Lin's group reported an innovative strategy of mild photothermal therapy (PTT) based on Pd SAzymes to promote

the treatment of ferroptosis.[165] The Pd SAzyme showed POD- and GSHOx-like activities and photothermal conversion performance, which could lead to the up-regulation of LPO and ROS. The accumulation of LPO and ROS provided a powerful way to split heat shock proteins (HSPs) which could repair cell damages caused by heat, making Pd SAzyme-mediated mild PTT possible.

In 2022, considering the effects of hyperthermia on the activity of SAzymes and the damage of cell tissue, Liu's group used $Bi_2Fe_4O_9$ nanosheets as an enzyme mimic by low-temperature activation (4 – 37 °C), which featured a wider safety window than those treated at higher temperatures (37 – 42 °C). The cold-activated SAzyme displayed GSHOx- and POD-like activity and mediated the antitumor treatment.[166] Notably, the GSHOx-like activity of the $Bi_2Fe_4O_9$ SAzyme could only be activated at low temperatures, whereas the POD-like activity of $Bi_2Fe_4O_9$ SAzyme in acidic media and the CAT-mimic activity in neutral and alkaline conditions were unaffected by temperature changes. Therefore, the GSHOx-like activity activated at low temperatures promoted the oxidation of GSH to generate H_2O_2. Then intrinsic H_2O_2 together with the H_2O_2 generated from GSH oxidation was decomposed by POD-like $Bi_2Fe_4O_9$ SAzymes. When the GSHOx-like activity was turned off by an interventional device (e.g., a smart phone), the enzyme activity could be remotely and precisely modulated, so as to eliminate excessive ROS through CAT-like activity and minimize damage to normal tissues. Similarly, Wu's group observed that a Ni SAzyme doped with sulfur and nitrogen via an anion exchange method showed better POD- and GSHOx-like activity than the undoped counterparts and could effectively inhibit the growth of tumor cells without toxicity (**Figure 11a**).[167]

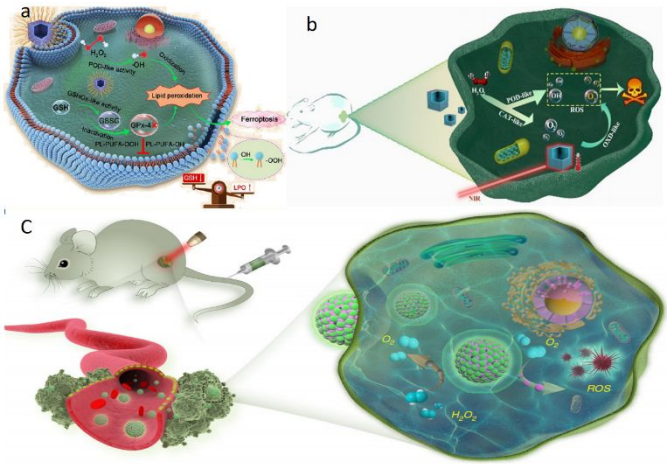


Figure 11. (a) Illustration of inhibiting tumor cell growth by inducing iron ferroptosis. Sulfurized S-N/Ni PSAE exhibits higher GSHOx-like activity than nitrogen-monodoped N/Ni PSAE for depleting intracellular GSH, resulting in the inactivation of GPx-4. Moreover, S-N/Ni PSAE with superior POD-like activity converts endogenous H_2O_2 into highly toxic •OH, causing LPO via oxidizing PUFAs. Reproduced with permission from ref. 167, copyright 2023, American Chemical Society. (b) Illustration of manganese-based single-atom enzyme for tumor therapy utilizing the synergetic catalytic and photothermal therapy. Reproduced with permission from ref. 171, copyright 2021, Wiley. (c) Scheme of continuously catalytic oxygen generation and ROS production for enhanced tumor photodynamic therapy by OxgeMCC-r SAzyme. Reproduced with permission from ref. 164, copyright 2020, Springer Nature.

As the tumor microenvironment features insufficient oxygen supply and excessive ROS (such as H_2O_2),[168] one can take advantage of the unique activity of SAzymes to catalyze the decomposition of H_2O_2 to cytotoxic hydroxyl radicals (•OH) and locally kill the tumor cells. This was indeed demonstrated recently by He et al. with a Pd SAzyme based on phenolic carbon quantum dot (DA-CQD@Pd).[169] Meanwhile, SAzymes can catalyze free-radical polymerization to form immune adjuvant CpG oligodeoxynucleotides (ODN), which can initiate catalytic immunotherapy to achieve localized immunomodulation and prevent tumor metastasis. Liu's group prepared an Fe SAzyme containing FeN_5 moieties embedded within N-rich carbon via a melamine-mediated two-step pyrolysis strategy,[55] where the FeN_5 active sites catalyzed the decomposition of H_2O_2 to • OH. Feng's group integrated Au nanoparticles into the pores of a dendritic mesoporous Fe SAzyme,[170] where the Au nanoparticles interacted with glucose to generate gluconic acid and H_2O_2 in a nanozyme-like manner, and H_2O_2 was further decomposed into ROS due to the POD-like activity of the Fe SAzyme. This kind of GOD-like activity can cut off the energy supply of the tumor cells, and in combination with photothermal agents (PTAs), achieve photothermal treatment of tumors at low temperatures.

Apart from the POD-like activity, many SAzymes possess multienzyme properties and can initiate cascade enzymatic reactions and enhance the therapeutic efficacy. Zhu et al. utilized hollow ZIF as a precursor to prepare a PEGylated manganese-based SAzyme (Mn/PSAE), which catalyzed the conversion of cellular H_2O_2 to •OH by the POD-like activity, as well as promoted the production of O_2 from H_2O_2 and the conversion of O_2 to $O_2^{\bullet-}$ via OXD-like activity.[171] The amorphous carbon substrate materials could enhance the PTT effect. In short, the enzyme cascade reactions initiated by SAzymes with multienzyme catalytic properties facilitate the continuous redox reaction of cells and the accumulation of cytotoxic ROS, which is a favorable and implementable pathway to enhance the anti-tumor effect (**Figure 11b**).

Despite such breakthroughs in SAzyme-based tumor therapy, there remains much room to improve the specificity. Studies have shown that the combination of nanomotor and near-infrared (NIR) technology can enhance adhesion of SAzymes to the cancer cell membrane and deepen penetration into the tumor.[172] Another desirable route is to combine chemodynamic therapy,[173] sonodynamic therapy[174] and photothermodynamic therapy[175] to improve targeting. One can envision that precise drug administration can be achieved when drugs aare loaded onto the SAzymes for targeted transport to the cancer sites.[176] In conclusion, targeted tumor therapy has remained a key clinical challenge, and SAzymes have emerged as a unique platform, which demands further and more thorough studies.

5.3 Antibacterial and antiviral activity

Bacterial infection is a grave threat to human health, which is compounded by the emergence of drug-resistant bacteria. ROS can act as powerful weapons against pathogen invasion by disintegrating the structure of biofilms, while avoiding the generation of drug-resistant strains due to effective damages of bacterial cells.[177, 178] SAzymes possess unique advantages as compared to traditional antibacterial agents, such as high

efficiency, low cost, good biocompatibility, non-cytotoxicity and adjustable enzyme activity, where the bactericidal mechanism is primarily based on in vivo decomposition of hydrogen peroxide or oxygen to ROS.[179]

Previous reports have shown that Cu SAzyme exhibited excellent antibacterial activity. Zhao et al. prepared a highly accessible Cu SAC supported on biocompatible N-doped mesoporous carbon nanospheres via an emulsion-template method.[180] The ultra-large pore size and small particle size of the mesoporous carbon nanospheres boosted the efficient conversion of O_2 to $O_2^{\bullet-}$, resulting in the damage of bacterial cell membrane and the splitting of the bacterial body. Wang et al. synthesized N-doped porous carbon-supported Cu SAzymes (Cu SASs/NPC) by a pyrolysis-etching-adsorption-pyrolysis (PEAP) strategy.[181] The Cu SAzymes possessed prominent POD-like activity in catalyzing the decomposition of H_2O_2 to •OH and GSH peroxidase (GSH-Px)-like activity to deplete GSH, and consequently a nearly 100% antibacterial efficacy against the two most common pathogenic bacteria, *Escherichia coli* (*E. coli*) and methicillin-resistant *Staphylococcus aureus* (*S. aureus*) through the synergistic effect of PTT (**Figure 12a**). Meng et al. utilized a unique support consisting of a nanodiamond core and curved graphene shells (ND@G) that was decorated with abundant defects to anchor Cu metal atoms, resulting in atomically dispersed and fully exposed Cu_3 clusters on the ND@G surface (**Figure 12b**).[182] This structure significantly enhanced the OXD-like activity in the dissociation of O_2 into •OH to kill bacteria.

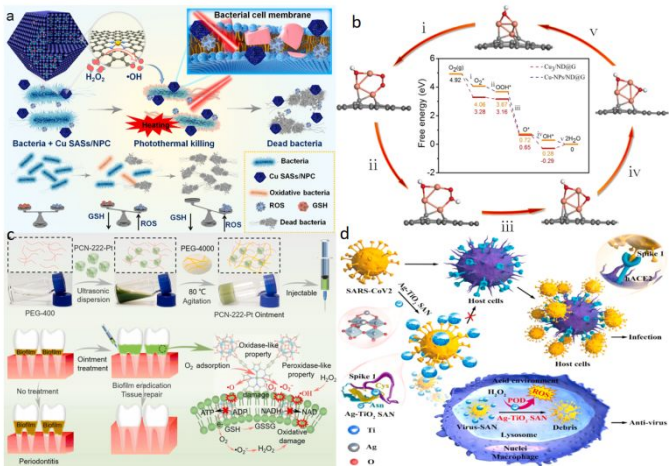


Figure 12. (a)Cu SASs/NPC with GSH-depleting performance for photothermal-catalytic therapy against bacterial. Cu SASs/NPC as GSH-like mimetic enzyme and HRP-like nanozyme for eradicating *E. coli* and *MRSA in vitro.* Reproduced with permission from ref. 181, copyright 2021, Elsevier. (b) Theoretical investigation of OXD-like activity over Cu-NPs/ND@G and Cu_3/ND@G. The optimized adsorption configurations of various intermediates along the oxidase-like reaction path on Cu_3/ND@G and the free energy diagram for the oxidase-like mechanism on Cu_3/ND@G and Cu-NPs/ND@G. The gray, brown, red and white balls represent C, Cu, O, and H atoms, respectively. Reproduced with permission from ref. 182, copyright 2022, Elsevier. (c) Preparation of the injectable HNTM-Pt ointment with enzyme-like catalytic activities and the treatment of periodontitis through a series of catalytic reactions. Reproduced with permission from ref. 184, copyright 2022, Elsevier. (d) Schematic of Ag-TiO_2 SAzymes with anti-SARS-CoV_2 activity. Ag-TiO_2 SAzymes adsorb onto the receptor binding domain (RBD) of spike 1 protein of SARS-CoV_2 containing abundant cysteine and asparagine to generate the SAzyme/virus complex, which can be phagocytosed by macrophages and colocalized with lysosomes. Reproduced with permission from ref. 185, copyright 2021, Elsevier.

In addition to copper, other metal SAzymes also show effective antibacterial activity. Yang's group prepared a g-ZnN_4-MoS_2 SAzyme via electrostatic interactions, which can be applied to sonodynamic ion therapy to upgrade the antibacterial efficacy.[183] Benefiting from the synergetic regulation of the Zn single-atom and MoS_2 quantum dots, charge transfer and spin-flip were enhanced at the heterogeneous interfaces, which promoted the generation of singlet $^1O_2$ from O_2 owing to a reduced activation energy of O_2. It has been shown that $^1O_2$ can kill *S. aureus* with an antibacterial efficiency of 99.58% under 20 min of ultrasound irradiation. The excellent sonodynamic antibacterial efficiency and osteoinduction ability of g-ZnN_4-MoS_2 suggested attractive applications in ultrasonic antimicrobe. Another similar instance is a Pt SAzyme modified porphyrin MOF (PCN-222-Pt), where the active sites exhibit strong OXD- and POD-like activities and can lower the desorption energy of O_2 and produce ROS (**Figure 12c**). It displays an excellent anti-biofilm performance to deactivate bacteria at an inactivation efficiency of *S. aureus* and *E. coli* of 98.69% and 99.91%, respectively [184].

SAzymes possess not only prominent bactericidal activity, but also play a vital role as high-efficiency catalysts in antiviral research. Notably, Wang et al.[185] prepared Ag-TiO_2 SAzymes to combat SARS-CoV_2 coronavirus, which is currently posing a serious threat to global public health. The materials were rationally designed through a combination of ingenious experimental engineering and theoretical calculations, and exhibited a high POD-like activity (**Figure 12d**). Scanning electron microscopy (SEM) measurements showed that the SAzyme adsorbed onto the receptor binding domain (RBD) of the spike 1 protein of SARS-CoV_2 that contained abundant cysteine and asparagine via strong interactions with the Ag centers. The SAzymne/virus complex was then phagocytosed by macrophages and colocalized with lysosomes which supplied an appropriate acid condition for enzyme activity. Compared with traditional TiO_2 or Ag nanomaterial, Ag-TiO_2 SAZyme exhibited a much higher adsorption efficacy (99.65%) of SARS-CoV_2 pseudovirus. This demonstrated the great potential of SAzymes in antiviral applications.

5.4 Other applications

SAzymes have also been successfully applied in food safety monitoring,[186-189] environmental survey,[190-193] cytoprotection,[194, 195] wound healing,[196] nervous system diseases therapy and brain trauma,[197-200] as well as drug dosing guides and outcome predictions. For instance, Ce-N-C SAzymes have been utilized for food safety monitoring and detection of pesticide residues, where the POD-like activity provided a colorimetric route to efficiently detect organophosphorus and carbamate pesticide residues via cascade reactions with AChE. The Ce SAzyme was integrated with bioactive paper using the 3D printing technology.[201] Mechanistically, TMB were oxidized to blue oxTMB, which was then reduced to colorless TMB by choline in the presence of Ce-N-C SAzyme and hydrogen peroxide. However, organophosphorus and carbamate pesticide residues could inhibit the activity of AChE and produce little or no choline, blocking the reverse reduction reaction. The color of bioactive paper was correlated with the pesticide residue content, allowing for the rapid portable detection of pesticides. Furthermore, Yang's group found that

hemin-loaded Zn-N-C SAzymes possessed an intrinsic POD-like activity, which could be used for rapid detection of propylgallate and formaldehyde in food samples.[202] The POD-like activity led to the oxidation of colorless propylgallate to yellow products while HCHO could inhibit propylgallate oxidation, leading to an apparent decrease in optical absorbance.

In addition, SAzymes exhibit antibacterial activity against a broad range of marine microorganisms and can act as promising anti-biofouling agents. Sun et al. developed a Co SAzyme with Co single atoms loaded into polyacrylamidoxime (PAO).[203] The Co-PAO SAzyme catalyzed the generation of ROS from hydrogen peroxide and destruction of marine fouling biological entities, such as marine bacteria and marine algae.

Furthermore, as the abundant ROS generated by oxidative stress can damage brain tissue and induce a series of neuroinflammation, leading to the occurrence of various central nervous system (CNS) diseases, select SAzymes have also been found to exhibit satisfactory ROS-scavenging activity and great potential for the treatment of CNS diseases.[204] For instance, ultrasmall carbon dots-supported Fe SAzyme (Fe-CDs) could serve as a kind of nontoxic nanomedicine to modulate tumor microenvironment via ROS regulation and lysosome-mediated autophagy.[205] It has six naturally occurring enzymatic properties, OXD, CAT, SOD, and the POD family (HRP, GPx, and thiol peroxidase), that induce a cascade of reactions in vivo. In addition, the blood-brain barrier permeability and the ability to selectively target GBM in vivo can be greatly enhanced by peptide modification on the surface of Fe-CDs. Benefiting from the multiple enzyme-mimic properties and minimal toxicity, the Fe-CDs SAzymes possess a great potential in precise therapy of drug-resistant glioblastoma (GBM). Recently, Zhang et al. observed an ultrahigh biological activity of RhN_4, VN_4, and $FeCuN_6$ SAzymes through precise atomic engineering. The RhN_4 and VN_4 SAzymes formed an Rh/V-O-N_4 active site, displaying 4- and 5-fold higher affinity in POD-like performance than the FeN_4 and natural HRP. The RhN_4 structure also showed a CAT-like activity that was 20 times higher than that of the natural enzyme, while the VN_4 structure possessed a high GPx-like activity 7 times higher than that of the natural counterpart. The $FeCuN_6$ also showed an excellent SOD-like activity. These catalytic activities can be combined to construct novel healing sutures for brain trauma by promoting the vascular endothelial growth factor, regulating the immune cells like macrophages, and diminishing inflammation.[206]

6. Conclusion and Perspectives

SAzymes are a rising star in the field of catalysis and biomedicine, primarily because of multiple catalytic activities that mimic those of POD, OXD, CAT, and SOD, which can mediate the redox reactions between various substances, generating or scavenging ROS. In comparison to the easy degradation and high costs of natural enzymes and complicated structures and limited catalytic activity of conventional nanoparticle-based nanozymes, SAzymes possesses excellent stability, well-defined central structure and low cost, leading to diverse applications in various fields. With homogeneously dispersed active sites and well-defined coordination structures of SAzymes, it has become possible to explore the correlation between the material structure and activity via rational regulation of the geometric and electronic structure. As highlighted in this review, there is no doubt that SAzymes, as emerging high-performance biocatalysts, will become promising alternatives to traditional enzymes for a range of biological/biomedical applications.

Yet, despite significant progresses, obstacles and challenges remain that hinder the further development of SAzymes. From the materials perspectives, most SAzymes are prepared by controlled pyrolysis of select precursors, where the carbon scaffolds typically exhibit a complex structure and aggregation of metal atoms inevitably occurs. The complexity of the materials structure renders it challenging to establish an unambiguous correlation between the materials structure and enzymatic activity. Thus, development of effective methods for the preparation of SAzymes with well-defined structures are urgently needed. This calls for a careful design of the precursors as well as deliberate manipulation of the thermal treatment conditions. Towards this end, ultrafast synthesis may be a viable option, where the ultrashort heating pulses can facilitate the retention of the organized structures of the precursors.[207] In addition, one may take advantage of the latest breakthroughs in organometallic chemistry to pre-position the metal centers within a molecular framework.[208] This will also be of particular importance when binuclear or even multinuclear atomic sites are desired.

From the biological perspective, while enzyme-like activities have been observed with SAzymes, the catalytic performance and substrate selectivity have remained mostly subpar as compared to natural enzymes. This is likely due to the high-order structure of the nanocomposites that deviates from the natural enzyme. With the advances of computing power and methods, the materials structure of the nanocomposite-based SAzymes can be optimized within the context of, for instance, covalency between the metal center and coordinating atoms, porosity of the structural scaffold, doping of heteroatoms, etc. In addition, thus far, the mechanistic insights into the interactions between SAzymes and biological targets have remained limited. This is particularly true with living microorganisms like bacterial cells and viruses, where the antimicrobial and antiviral activity may be a combined contribution of damages of the cell structural integrity and inhibition of the metabolism. In vivo imaging and spectroscopy analysis will be a key to unravelling the mechanistic details, which can be aided by low-temperature electron microscopy measurements.[209, 210]

Author Contributions

Xueqian Xiao: Investigation, Writing – original draft; **Xiao Hu**: Investigation, Writing – original draft; **Qiming Liu**: Writing – review & editing; **Yulin Zhang**: Writing – original draft; **Guo-Jun Zhang**:

Resources, Writing – original draft; **Shaowei Chen**: Conceptualization, Resources, Writing – Review & editing.

Conflicts of interest

There are no conflicts to declare.

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