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Graphene-based nanozymes for revolutionizing biomedical research

 Astha Tripathi,^{ab} Saumya Singh Rautela^b and Pratibha Kumari ^{*b}

Nanozymes, with nanoscale dimensions and enzyme-like properties, have garnered substantial interest as they can overcome the downsides of conventional enzymes, including fragility, high cost, tedious isolation, and expensive manufacturing. Over the past decade, a diverse range of nanomaterials have demonstrated the ability to mimic enzyme-like activity by incorporating multivalent elements into nanostructures. Notably, graphene-based nanomaterials, such as graphene, graphene oxide, and reduced graphene oxide, with their precisely controlled scaffolds and electronic properties, have emerged as promising substitutes for traditional enzymes by emulating the intricately evolved catalytic centers of natural enzymes, including oxidase, peroxidase, catalase, and superoxide dismutase. The distinct electronic, mechanical, thermal, and optical properties enable graphene-based nanozymes to provide multifunctional platforms for various biomedical applications, including wound healing, tissue regeneration, cancer treatment therapies, antibacterial activity, and biosensing applications. This in-depth review examines the enzymatic features and recent advancements of graphene-based nanozymes, highlighting their significant contributions to biomedicine.

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1. Introduction

The field of nanomaterials has witnessed unprecedented growth, resulting in the burgeoning of novel materials with exceptional attributes and promising applications across various scientific and technological domains.¹ One such intriguing area of focus in nanomaterial research is the utilization of nanozymes. A landmark discovery in 2007 challenged the long-held belief that inorganic materials are biologically inactive. This motivated researchers to investigate the catalytic potential of nanomaterials as “nanozymes”. Nanozymes refer to a new generation of artificial enzymes that have revolutionized the landscape of biomedical research by emulating the function of natural enzymes at the nanoscale.² These are promising areas of research at the intersection of nanotechnology and biology.^{3,4}

Enzymes are highly efficient biocatalysts, enabling numerous chemical reactions in living organisms and playing a crucial role in various biological processes. Enzymes accelerate biochemical reactions, enabling the efficient operation of various metabolic pathways, cellular processes, and physiological functions. Enzymes exhibit high catalytic activity, high substrate specificity, and selectivity under mild conditions. However, natural enzymes have limitations, such as instability, high cost, tedious purification, and storage, which restrict their

widespread technological applications.^{5–7} Nanozymes, on the other hand, offer several advantages over natural enzymes, such as high stability, low cost, and ease of production. Furthermore, nanozymes can be tuned to have specific catalytic properties, making them extremely versatile and suitable for numerous applications.^{8–10} A vast array of nanozymes is currently employed in biosensing and therapeutics. Nanozymes are usually metal and metal oxide nanoparticles,^{11,12} metal–organic frameworks,¹³ two-dimensional materials,¹⁴ inorganic nanomaterials,¹⁵ covalent-organic frameworks,¹⁶ transition metal-based nanomaterials,¹⁷ carbon-based nanomaterials,¹⁸ biomolecules,¹⁹ and supramolecules.²⁰ These nanozymes exhibit exceptional catalytic activities and are being increasingly recognized for their potential to revolutionize the fields of biosensing and catalytic therapy, as summarized in Table 1.

The most promising candidates for developing nanozymes are graphene-based materials such as graphene, graphene oxide (GO), and reduced graphene oxide (rGO) (Fig. 1). Graphene and graphene-based materials are extensively studied nanomaterials with exceptional promise for a range of applications, including biomimetics and heterogeneous catalysis, due to their abundant chemical adaptability, remarkable stability, low toxicity, metal-free nature, and notable electrical and optical properties.⁴⁴ The functionalization of heteroatoms and metallic or bimetallic nanoparticles enables these nanozymes to closely replicate the active sites of specific enzymes, thereby enhancing their catalytic efficiency and selectivity in biochemical reactions. Their robust structure allows them to maintain activity over extended periods, making them more reliable for long-

^aDepartment of Chemistry, University of Delhi, New Delhi-110007, India

^bBioorganic Material Research Laboratory, Department of Chemistry, Deshbandhu College, University of Delhi, Kalkaji, New Delhi-110019, India. E-mail: pkumari@db.du.ac.in


Table 1 Overview of various conventional nanozymes, their enzymatic activities, and applications in biomedical fields

Nanomaterial	Activity	K_m (H_2O_2)	V_{max} (H_2O_2)	Biomedical applications	References
HCS@Pt-Ce nanoparticles	Peroxidase	0.04853 mM	$0.82 \times 10^{-8} M s^{-1}$	Photodynamic catalytic tumor therapy	21
Sm-TCPP-Pt/TPP nanosheets	Catalase	—	—	Photodynamic therapy	22
PVP-PCuNCs	Superoxide dismutase, catalase, and peroxidase	9.94 mM (Cat) 75.55 mM	$26.16 \times 10^{-6} M$ per s (Cat) $35.88 \times 10^{-8} M s^{-1}$	Hydroxyl radical scavenging	23
TiO ₂ nanotubes@MoS ₂ nanoflowers	Peroxidase	0.085 mM	$12.05 \times 10^{-7} M s^{-1}$	Wound healing	24
Hemin/graphdiyne (GDY) nanocomposite	Peroxidase	6.1 μ M	$1.14 \times 10^{-7} M s^{-1}$	Wound healing, antibacterial activity	25
Ag NC	Oxidase	119 μ M	$214 \times 10^{-9} M s^{-1}$	Hg(II) sensing	26
Pt NP	Oxidase	0.09 mM	$7 \times 10^{-6} M s^{-1}$	Heparin detection	27
Pd NC	Oxidase	—	—	Antitumor	28
Au@Pt	Oxidase, and peroxidase	0.027 mM	$181 \times 10^{-9} M s^{-1}$	Immunoassays	29
NiCo ₂ O ₄	Oxidase, and peroxidase	9.406 mM	$25.84 \times 10^{-8} M s^{-1}$	Glucose detection	30
CoOOH	Phosphatase and oxidase	—	—	Alkaline phosphatase	31
MoO ₃	Phosphatase and oxidase	1.6769 mM	—	Acid phosphatase	32
V ₂ O ₅	Oxidase	—	—	Glutathione sensing	33
Mn ₃ O ₄	Oxidase	0.025 mM	$5.07 \times 10^{-8} M s^{-1}$	Antioxidant activity of phenols	34
Co ₃ O ₄	Oxidase	0.037 mM	$3.20 \times 10^{-8} M s^{-1}$	Sulfite detection	35
Ag ₂ O	Oxidase	—	—	Sulfite detection	36
Tb ₄ O ₇	Oxidase	0.124 mM	$4.31 \times 10^{-8} M s^{-1}$	Antibacterial	37
IrO _x	Oxidase and peroxidase	19.27 mM	$1.79 \times 10^{-6} M s^{-1}$	Antitumor	38
Mixed valence state Ce-MOF	Oxidase	0.37 μ M	$5.5 \times 10^{-6} M s^{-1}$	Biothiols sensing	39
PVP-MoS ₂	Peroxidase	3.66 mM	$4.76 \times 10^{-8} M s^{-1}$	H ₂ O ₂ & glucose detection	40
CQDs/Fe ₃ O ₄	Peroxidase	56.97 mM	$5.744 \times 10^{-8} M s^{-1}$	H ₂ O ₂ & ascorbic acid detection	41
V ₂ N ₃ Ss	Peroxidase	3.49 mM	$55.7 \times 10^{-8} M s^{-1}$	Glucose detection	42
AuNR@TiO ₂	—	$0.20 \pm 0.02 \mu$ M	—	Catalysis	43



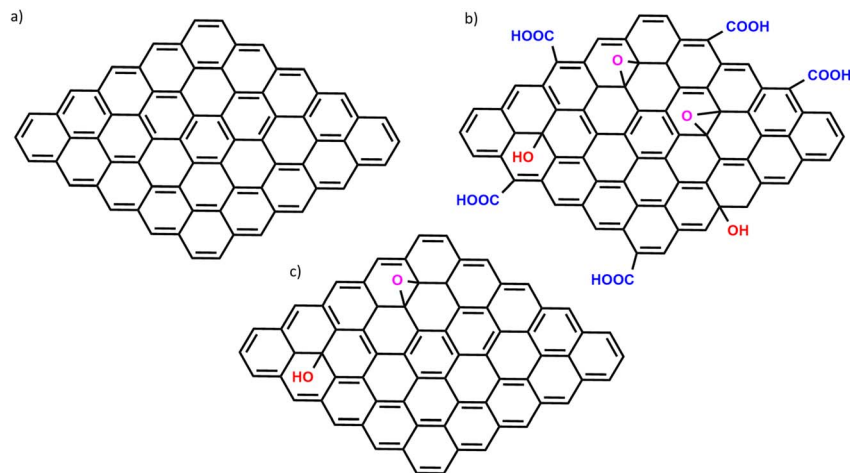


Fig. 1 Structures of (a) graphene, (b) graphene oxide, and (c) reduced graphene oxide.

term applications in biomedical fields, including wound healing, cancer cell detection, bioimaging, and therapeutics.⁴⁵ This capability is crucial in developing biosensors and therapeutic agents that require precise activity under physiological conditions. Apart from stability, graphene-based nanozymes depict enhanced electron transfer kinetics. This property facilitates rapid reactions with reactive oxygen species (ROS), which are often implicated in inflammation and tissue damage during wound healing processes. By effectively scavenging reactive oxygen species (ROS), graphene-based nanozymes can mitigate oxidative stress and promote a more favorable microenvironment for healing.^{46,47} The multifunctionality of graphene-based nanozymes also sets them apart from other nanozyme materials. They can serve as scaffolds for cell proliferation and migration while providing antimicrobial properties. For example, studies have shown that graphene-reinforced scaffolds enhance fibroblast migration and effectively inhibit bacterial growth, thereby supporting wound closure and preventing infection.⁴⁸ This dual functionality is less commonly observed in other nanozymes, which may focus solely on antimicrobial action or tissue regeneration but not both. The advantages and disadvantages of graphene-based nanozymes are shown in Fig. 2. By accelerating the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) in the presence of hydrogen

peroxide (H_2O_2), graphene-based nanozymes exhibit peroxidase-like activity and produce a blue-colored product. This catalytic process involves activation of oxygen-containing functional groups, particularly carbonyl groups, which serve as active centers for biocatalytic activity. The mechanism begins with the interaction of H_2O_2 with these functional groups, leading to the generation of reactive species that facilitate the oxidation of TMB.

Although graphene-based nanozymes do not strictly follow the traditional ping-pong mechanism, they exhibit a similar stepwise process where the activation of the carbonyl bond is crucial for the catalytic cycle. The high surface area and electron transfer capabilities of graphene-based materials enhance their reactivity, enabling them to mimic natural peroxidases effectively.²⁶ The graphene-based nanozymes have created highly sensitive and selective biosensors capable of detecting various biomarkers. In cancer diagnosis and therapy, peroxidase mimetic nanozymes have been extensively studied for their role in catalytic cancer therapy, complementing traditional treatments such as chemotherapy, phototherapy, and immunotherapy.^{49,50} The application of nanozymes in cancer treatment is multifaceted. They can kill cancer cells directly by enhancing reactive oxygen species (ROS) levels or indirectly by depleting ROS, thus exploiting the tumor microenvironment's

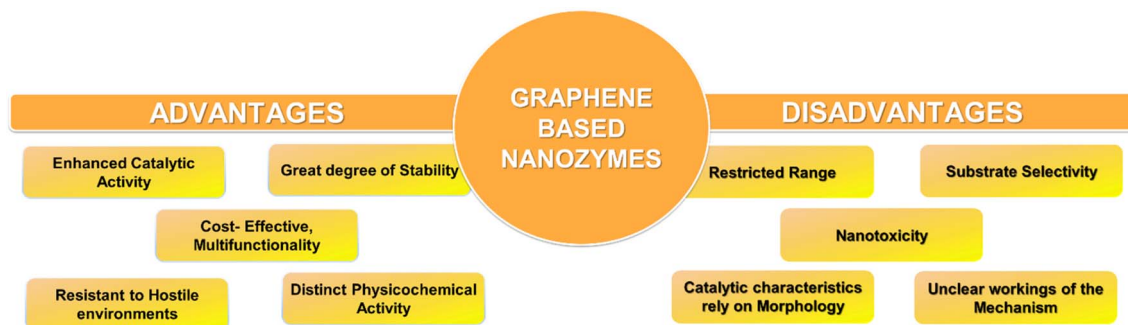


Fig. 2 Advantages and disadvantages of graphene-based nanozymes over conventional enzymes.



characteristics, such as hypoxia. Nanozymes' catalytic activity is influenced by various factors, including pH, substrate concentration, and temperature, which can be optimized for specific therapeutic outcomes.

Recent advancements in graphene-based nanozymes have expanded their applications to include the detection of glucose, dopamine, cholesterol, and various disease-related biomolecules. These sensors often employ functionalization techniques to enhance their performance, using both covalent and non-covalent approaches to modify graphene for specific sensing applications.⁵¹ For instance, graphene oxide functionalization has yielded graphene-based nanozymes that closely mimic several natural enzymes.⁵² Graphene-based nanozymes are appealing due to their ease of synthesis and retention of catalytic activity even after prolonged storage at ambient temperature.⁵³ The peroxidase-mimicking activities of graphene oxide and reduced graphene oxide have been widely reported, and they have been applied to the colorimetric detection of H₂O₂ and other substrates.⁵⁴ The peroxidase-like activities of rGO have also been selectively enhanced by N-doping in carbon nanomaterials, most likely due to the adjustment of charge density and an increase in active sites. The origin of the peroxidase-mimicking activity of graphene-based nanomaterials remains unclear. Some studies indicate that the carbonyl groups are the active centers among the various oxygen moieties. Several graphene-based hybrids have been developed as nanozymes and applied to construct electrochemical biosensors involving GO-COOH and GO-COOH hybrids.⁵⁴ Graphene-based nanozymes are at the forefront of innovation in biosensing and catalytic cancer therapy. Their unique properties have opened new avenues for developing sophisticated therapeutic and diagnostic tools. Wang *et al.* fabricated oxygen-functionalized graphene quantum dots (o-GQDs) that exhibit peroxidase enzymatic activity, surpassing that of other nanozymes.⁵⁵

Interest in the enzyme-mimicking properties of graphene nanomaterials began to gain traction around 2010, with early speculation and foundational studies highlighting their potential as artificial enzymes. The first report explicitly describing graphene oxide nanosheets as peroxidase mimics appeared in 2010, where carboxyl-modified graphene oxide was shown to catalyze reactions similar to horseradish peroxidase, leading to applications such as glucose biosensing.⁵⁶ The term “graphene nanozyme” was first introduced in the literature in 2011, marking a formal recognition of this new class of catalytic nanomaterials. Following these initial discoveries, research activity in the field was limited, with no publications recorded in 2012. However, after 2015, the number of studies on graphene-based nanozymes increased exponentially, reflecting the growing recognition of their versatility and catalytic efficiency. Functionalization of graphene, such as doping with nitrogen, boron, or metallic nanoparticles, has been shown to significantly enhance its enzyme-like activities, particularly for peroxidase, oxidase, and superoxide dismutase-mimetic reactions, and to improve selectivity and catalytic performance. The rapid expansion of this field is evident in bibliometric data: as of May 27, 2025, a search on CAS SciFinder reveals that there are

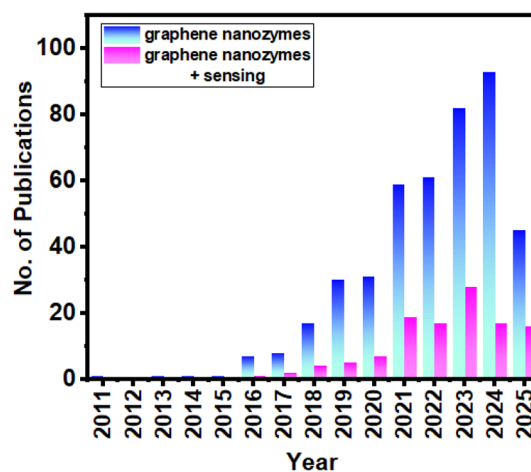


Fig. 3 Number of publications in journals including the keywords “graphene, nanozyme” and “graphene, nanozyme + sensing” from 2011 to 2025 (accessed on May 27, 2025 using CAS SciFinder search tool).

currently 437 publications related to “graphene” and “nanozymes,” including 388 research articles, 25 reviews, and several conference and editorial pieces. Of these, 118 specifically address applications in sensing, underscoring both the breadth of research and the diverse, promising prospects of graphene-based nanozymes in biosensing, catalysis, and environmental remediation. This trajectory highlights the transition of graphene nanozymes from a speculative concept to a vibrant and multidisciplinary research area (Fig. 3).

The objective of this review is to provide an in-depth understanding of the synthesis as well as applications of graphene-based nanozymes in wound healing, biosensing, antibacterial, and catalytic therapy. By examining the enzymatic characteristics, recent advancements, and innovative strategies employed in the development of graphene-based nanozymes, this review aims to elucidate their pivotal role in revolutionizing the field of biomedical research.

2. Synthesis of graphene and graphene-based nanozymes

Graphene and graphene-based nanozymes can be synthesized using several methods (Fig. 4), each offering distinctive advantages and applications. The different synthetic techniques can be precisely regulated to confer properties for targeted and intended applications.⁴⁴ The bottom-up approach is often used for graphene synthesis, involving smaller molecules such as carbon atoms or hydrocarbons, through a series of chemical reactions. This method enables the control of graphene's morphology, crystallinity, and structure by adjusting the chemical composition and reaction conditions. The bottom-up approach is appealing for producing high-quality graphene with tailored characteristics, including excellent and consistent thickness. This method employs techniques such as chemical vapor deposition (CVD) and pyrolysis, which utilize carbon precursors in a gaseous form for deposition onto a substrate.



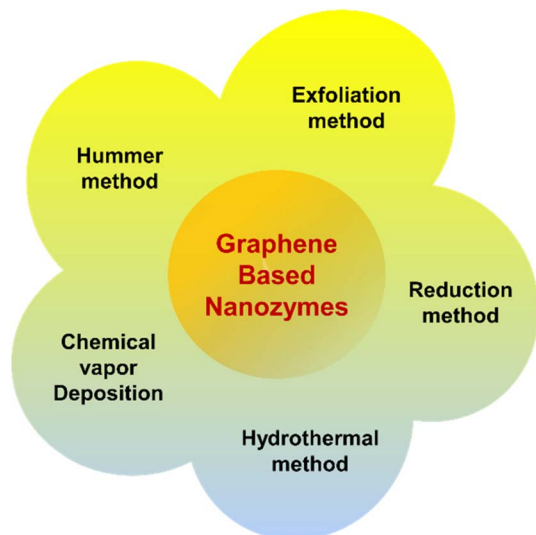


Fig. 4 Various synthesis methods to produce graphene-based nanozymes.

On the contrary, the top-down approach produces graphene flakes or sheets by mechanically or chemically exfoliating graphite, a common precursor to graphene. This method is often used for bulk graphene production due to its cost-effectiveness and ease of scaling up. The top-down approach can result in a broader range of graphene morphologies and properties, including polycrystalline films and flakes of varying sizes. This method involves chemical oxidation-reduction, liquid-phase exfoliation (LPE), and electrochemical exfoliation. This method simplifies the process by directly converting graphite to graphene. Still, it can lead to challenges such as surface defects during sheet separation and the re-agglomeration of separated sheets. Each approach has its advantages and disadvantages, and the preferred method often depends on the intended application and the specific properties of the graphene.

Chemical methods, such as Hummer's method, are commonly used to produce graphene oxide, providing a versatile platform for developing nanomaterials with inherent enzymatic properties. This method involves the oxidation of graphite using a mixture of concentrated sulfuric acid, sodium nitrate, and potassium permanganate, followed by sonication and filtration.⁵⁷ Hummer's method was further modified to enhance the nanozymatic activity. Kim *et al.* synthesized Fe-NRGO utilizing modified Hummer's method for the H₂O₂ detection.⁵⁸ Kumari *et al.* also synthesized graphene oxide using a modified Hummer's method.^{59,60} The modified Hummers' method offers significant advantages over conventional methods, primarily by eliminating sodium nitrate. This change is crucial as it can avoid the generation of toxic gases such as nitrogen dioxide (NO₂) and dinitrogen tetroxide (N₂O₄). Additionally, altered reactant ratios were employed to oxidize graphite to graphene oxide, such as increased amounts of KMnO₄ or other oxidizing agents, like potassium persulfate, which is often used to enhance oxidation efficiency and yield.

Another modification involved the use of phosphoric and sulfuric acid, which improved oxidation efficiency and safety.⁶¹ Phosphoric acid acts as an auxiliary oxidant, enhancing the oxidative chemical exfoliation of graphite when used in conjunction with sulfuric acid. This synergistic effect enables more effective intercalation and oxidation of graphite, resulting in a higher yield of graphene oxide. It also helps maintain a strong acidic environment, crucial for activating potassium permanganate, the primary oxidizing agent used in the process. This results in a more efficient oxidation reaction than methods that rely solely on sulfuric acid. This modification makes the process safer and more environmentally friendly.⁶² Lu *et al.* fabricated a surface-modified graphene-based hybrid as nanozymes, using the modified Hummer's method to synthesize graphene oxide.⁶³ Another method commonly used is the hydrothermal approach, primarily employed for synthesizing reduced graphene oxide. The degree of reduction is controlled by temperature; higher temperatures facilitate a more rapid reduction of graphene oxide.⁶⁴ With this technique, metal precursors (such as Fe³⁺ or Pt²⁺) react with graphene-based nanomaterials in an aqueous medium at high pressure and temperature to produce graphene-based nanozymes. This method often yields metal-graphene composites that exhibit excellent catalytic activity.^{65,66}

Li *et al.* developed gold adsorbed on graphene sheets to exhibit peroxidase-mimic activity, which was synthesized using a pot-hydrothermal method.⁶⁷ The reduction method is another top-down approach for synthesizing graphene-based nanozymes. Chemical reduction, thermal reduction, and electrochemical reduction are the approaches to synthesizing graphene-based nanozymes. The most prevalent method is the chemical reduction method, which involves reducing agents like hydrazine, sodium borohydride, ascorbic acid, and various plant extracts. This process effectively removes oxygen functional groups from the graphene oxide structures, converting them into reduced graphene oxide (rGO). The resulting rGO exhibits enhanced catalytic activity, resembling the behavior of nanozymes, which expands its potential applications across diverse fields. Liu *et al.* employed a green approach to synthesize a graphene-based nanozyme exhibiting peroxidase-like activity, which is also used for detecting L-cysteine.⁶⁸ In their study, polysaccharides served as the reducing agent for graphene oxide, facilitating an environmentally friendly reduction process. This innovative method enhances the catalytic properties of the resulting nanozyme, underscoring the potential of using biocompatible materials to synthesize functional nanomaterials for various applications. The thermal reduction method is another approach to reducing graphene oxide, which involves heating it to high temperatures in a controlled environment. The significance of this process lies in the elimination of oxygen functional groups and the re-establishment of the sp² carbon network, resulting in the formation of reduced graphene oxide (rGO), the active nanozyme form of graphene oxide. This is typically done inside the furnace, purged with an inert gas, and offers the most significant degree of freedom in terms of adjusting and maintaining the heating temperature and duration required to develop the desired enzyme-like properties.



Careful control of such parameters is essential primarily to enhance the catalytic activity of the resulting rGO, making it beneficial for various applications, including catalysis and biosensing. Another well-known approach for synthesizing graphene-based nanozymes is leveraging graphene's unique properties for catalytic purposes. This technique is classified into solid, liquid, and electrochemical exfoliation.⁶⁹ In electrochemical exfoliation, a direct voltage is applied to graphite in an electrolyte solution. Individual graphene sheets are expanded and exfoliated by gaseous species that are generated when ionic species are forced to intercalate into the graphite layers. This process has advantages in scalability and efficiency, allowing for the production of high-quality graphene with minimal defects. Furthermore, exfoliating graphene in the liquid phase is both cost-effective and environmentally friendly. Expensive and toxic solvents are avoided in this process. The resulting graphene sheets can be modified to enhance their catalytic properties, emulating those of enzymes, making them suitable for a wide range of applications.

One of the prominent methods for synthesizing graphene-based nanomaterials is solvothermal synthesis, which involves reacting precursors in a solvent at elevated temperatures and pressures. This method facilitates the formation of functionalized graphene-based nanozymes while offering scalability and versatility. However, it can lead to agglomeration effects that may affect performance. For instance, Li *et al.* fabricated Fe–N–GQD using a green and facile solvothermal approach.⁷⁰ This process treated the precursor with DMF, and the resulting solution was transferred to a PTFE liner. It was then heated at 180 °C for 9 hours. After synthesis, the product underwent purification through multiple cycles of centrifugation and dialysis. Borthakur *et al.* also employed the solvothermal technique to synthesize CuS and NiS nanoparticle-decorated porous reduced graphene oxide sheets, which demonstrated peroxidase-mimicking activity. They further utilized this for the detection of Hg²⁺ ions.⁷¹

Chemical vapor deposition (CVD) is a widely adopted bottom-up approach for synthesizing graphene-based nanozymes, as it produces high-quality graphene with controlled properties.⁷² In CVD, carbon-containing gases, such as methane, are decomposed at high temperatures in the presence of a substrate, typically copper or nickel, which facilitates the growth of graphene layers. This method enables precise control over layer thickness and doping levels, making it suitable for creating functionalized graphene that can enhance enzyme-mimicking activity. One of the significant advantages of CVD is its scalability; it can produce large-area graphene films essential for various applications, including nanozymes. Furthermore, CVD can incorporate dopants, such as nitrogen, during the growth process, which can tailor the electronic properties of graphene and enhance its enzymatic performance. For instance, nitrogen-doped graphene synthesized *via* CVD has demonstrated enhanced charge carrier mobility and stability, making it an excellent candidate for nanozyme applications.^{73,74} Additionally, CVD enables the synthesis of graphene in a relatively clean environment, minimizing impurities that could affect the performance of the resulting nanozymes. However,

challenges remain, such as achieving uniformity across large areas and managing production costs. CVD is a powerful technique for developing advanced graphene-based nanozymes with promising applications in biosensing and catalysis.

The choice of synthesis method depends on the intended application of the graphene-based nanozymes. Despite its higher cost and complexity, the CVD method is superior for applications that require high-quality materials with precise control over their properties. However, hydrothermal synthesis or electrochemical reduction may be more suitable for larger-scale production where cost-effectiveness is a priority, balancing quality and scalability. Each method has its unique advantages and challenges, making it essential to tailor the synthesis approach to the specific needs of each application.

2.1 Side product formation in graphene-based nanozyme synthesis and its impact on catalytic mechanisms

The synthesis of graphene-based nanozymes inherently involves the formation of various side products and impurities, which arise from the complexity of the chemical processes and the nature of the precursors used.^{75,76} For example, hydrothermal synthesis employing dopants like urea and boric acid for nitrogen and boron co-doping or graphene oxide combined with zinc acetate dihydrate for ZnO/GO composites often leaves residual unreacted starting materials. These residuals can interfere with the nanozyme's structural uniformity and catalytic properties. Additionally, the formation of multi-layer graphene, such as five-layer graphene observed in nitrogen–boron doped graphene (NB-Gr), and variations in reduction or doping levels introduce structural non-uniformity that affects catalytic site distribution and electron transfer pathways.⁷⁷

In chemical vapor deposition (CVD), a common side product is amorphous carbon contamination, often called carbon soot, which physically obscures the graphene surface and lacks the ordered structure necessary for catalytic activity. This contamination arises from uncontrolled carbon supply or high defect densities during growth and can be partially mitigated by hydrogen-assisted conversion to graphene. Residual oxidizing impurities like oxygen or moisture in the reactor atmosphere can etch the graphene film, creating defects and oxidized carbon clusters that degrade catalytic performance.⁷⁸ Moreover, incomplete removal of reaction by-products, unreacted gases, or residual metal catalysts (*e.g.*, nickel, cobalt, copper) used in CVD can remain on the graphene surface, further impacting catalytic efficiency.⁷⁹

Graphene-based syntheses often suffer from persistent oxygen-containing functional groups (hydroxyl, carboxyl, carbonyl, epoxy) due to incomplete reduction. While some oxygen groups can enhance catalytic activity by providing active sites or improving hydrophilicity, excessive or uncontrolled oxygen functionalities can initiate photodegradation and reduce long-term stability. Reducing agents like hydrazine or sulfur-containing compounds may also leave residual impurities if purification is insufficient.⁸⁰ These side products have a dual impact on catalytic mechanisms. Detrimental impurities such as amorphous carbon, excessive oxygen groups, or residual



metals can block active sites, impede electron transfer, and cause structural degradation, thereby lowering catalytic efficiency and selectivity.^{81,82} Conversely, intentional heteroatom dopants (nitrogen, boron, sulfur) and integrated transition metals serve as engineered “impurities” that create or enhance active sites, redistribute electron density, and optimize catalytic pathways, significantly boosting nanozyme activity.⁸³

Therefore, the challenge lies in precise impurity engineering—rigorously eliminating harmful side products while selectively introducing beneficial dopants. Advanced characterization techniques like Raman spectroscopy,⁸⁴ X-ray photoelectron spectroscopy (XPS), and transmission electron microscopy (TEM) are critical for identifying impurity fingerprints, guiding synthesis optimization, and ensuring reproducible catalytic performance. Ultimately, balancing purity with controlled defect and dopant incorporation is essential for designing graphene-based nanozymes with tailored catalytic properties suitable for practical applications, especially in complex biological environments where stability and specificity are paramount^{85,86}

3. Properties, tunability, and active sites of graphene-based nanozymes

Graphene-based nanozymes have emerged as a transformative class of nanomaterials that effectively mimic the activity of natural enzymes, offering tunable properties and diverse enzymatic functions suitable for various applications, including therapeutics and environmental remediation. Graphene-based nanozymes exhibit a range of catalytic activities, including peroxidase, oxidase, superoxide dismutase,⁸⁷ and catalase activities.⁸⁸ Various nanomaterials and molecular structures serve as excellent enzyme mimetics and nanozymes in various transformations.^{89–95} However, the tunability of graphene-based nanozymes represents a significant advancement in artificial enzymes, offering unprecedented control over catalytic properties and expanding their potential applications across various industries. Tunability in these nanozymes refers to the ability to modify and control their catalytic activity, selectivity, and stability through various structural and compositional adjustments. These activities can be modulated by altering the

graphene material's surface chemistry and electronic properties, allowing it to perform complex biochemical reactions. This tunability is achieved through several methods, including surface functionalization, the choice of reducing agents during synthesis, nanoparticle size and shape control, the incorporation of metal nanoparticles, and the application of external stimuli such as ultrasound or electromagnetic fields.

Unlike the pre-formed, fixed amino acid pockets characteristic of natural enzymes, the active sites in graphene-based nanozymes are not singular, static entities. Instead, they are often dynamically engineered features on the material's surface, arising from a sophisticated interplay of its intrinsic composition, defects, and deliberately introduced structural modifications. These sites collectively form a tunable catalytic landscape. The catalytic mechanism of graphene-based nanozymes involves the adsorption, activation, and electron transfer of the substrate at carbon surfaces. Unlike natural enzymes that rely on specific binding pockets, nanozymes often utilize surface-mediated interactions, including electrostatic or hydrophobic forces, for substrate binding.

Surface functionalization, for instance, involves modifying the graphene surface with different chemical groups or molecules to enhance its interaction with specific substrates or improve its catalytic properties.⁹⁶ Introducing oxygen-containing functional groups in graphene oxide (GO), a graphene derivative, enhances its dispersibility in aqueous solutions, which is beneficial for catalytic applications in biological and environmental systems. Graphene-based nanozymes host abundant active sites primarily comprising oxygen-containing functional groups, including hydroxyl (–C–OH), carbonyl (C=O), and carboxyl (O=C–O–) groups. These surface/defect-bound groups interact with substrates *via* hydrogen bonding or serve as catalytic centers, forming stable active sites. For example, carbonyl groups are key active centers for peroxidase-mimicking activities, with the activation of the C=O bond being a critical step in the catalytic cycle. Similarly, carboxyl functional groups have been identified as catalytic sites for the reduction of hydrogen peroxide (H₂O₂) to hydroxyl radical, and also function as substrate-binding sites for H₂O₂ (Fig. 5a).

Smaller nanoparticles with larger surface-area-to-volume ratios exhibit higher enzyme-like activity due to the presence

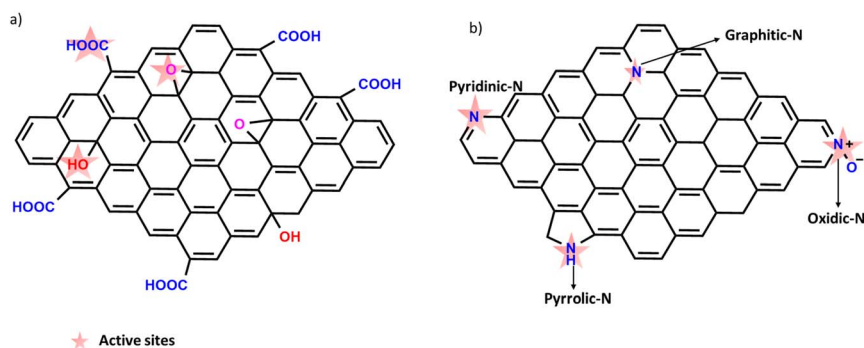


Fig. 5 (a) Active sites present on graphene oxide nanozymes. (b) Cluster model depicting four distinct nitrogen configurations in nitrogen-doped reduced graphene oxide (N-rGO) that serve as active sites.



of more unsaturated sites, although stability can be a concern. Encapsulation with a few-layer graphene can protect these particles, maintaining high catalytic activity and stability. Graphene quantum dots (GQDs) are particularly noteworthy, representing the latest addition to the nanocarbon family, characterized by their zero-dimensional structure and atomic-level thickness.⁹⁷ GQDs are utilized in sensing, anti-counterfeiting, energy storage, and cancer catalytic therapy due to their unique luminescent properties and excellent water dispersibility, which prevent aggregation in aqueous solutions. Two primary approaches enhance the catalytic efficiency of GQD-based nanozymes: optimizing the number of oxygen-containing functional groups and employing heteroatom doping or combinations with metal-based materials. For example, carboxyl and carbonyl groups on GQDs can serve as substrate binding sites and catalytic centers.⁹⁸ In a recent study, graphene quantum dots were synthesized using multiwalled carbon nanotubes (MWCNTs) as carbon precursors and concentrated nitric acid as an oxidizing agent through a simple oxidation reflux method. The resulting GQDs exhibited a surface enriched with carbonyl and carboxyl groups, while containing minimal hydroxyl groups, which significantly enhanced their peroxidase-like activity across a wide pH range.⁵⁵ Kinetic analyses further demonstrated that the GQDs had a Michaelis constant (K_m) value five times lower than other types of GQDs and an order of magnitude lower than horseradish peroxidase, indicating superior catalytic efficiency. These findings underscore the potential of GQDs in advancing nanozyme research through accessible synthesis and effective surface modification techniques.

Another approach for fine-tuning catalytic activities tailored to specific applications was the functionalization of graphene and graphene-based nanomaterials with metal oxides and heteroatom doping. The strategic incorporation of non-metallic heteroatoms, particularly nitrogen (N), but also boron (B), sulfur (S), and phosphorus (P), into the graphene lattice is a predominant and highly effective strategy for creating and significantly enhancing active sites. Nitrogen doping leads to the formation of various distinct nitrogen functionalities within the graphene structure, including pyrrolic-N, pyridinic-N, graphitic-N, and oxidized-N species (Fig. 5b). Each configuration contributes uniquely to the material's properties and catalytic activity. For example, pyridinic N is known for its higher coordination ability, enhanced transition metal loading capacity, and superior redox activity, while graphitic N offers higher electronic conductivity and structural stability. These specific nitrogen configurations are crucial as they serve as direct active catalytic sites for various reactions, including H_2O_2 catalysis. The incorporation of these heteroatoms into the graphene lattice induces a significant delocalization and redistribution of electronic states, which in turn modulates the material's physicochemical properties, creating highly active sites, often through bridging interactions with adjacent carbon atoms. This doping strategy dramatically increases catalytic activity; for instance, nitrogen-doped reduced graphene oxide (N-rGO) can exhibit catalytic activity several tens to hundreds of times greater than pristine rGO. Furthermore, synergistic

effects from co-doping, such as with nitrogen and boron, can lead to even more remarkable enhancements, with a reported 1000-fold increase in catalytic activity for H_2O_2 .

Integrating different metal oxides, such as Fe_3O_4 , MnO_2 , and CeO_2 , and heteroatoms into graphene structures significantly boosts enzymatic activity, providing a means to adjust functionality for therapeutic purposes. A highly effective approach to boosting catalytic performance involves the synergistic integration of transition metals with graphene-based materials, forming sophisticated hybrid active sites. Transition metals such as iron (Fe), copper (Cu), zinc (Zn), and cobalt (Co) can be strategically integrated within nitrogen-doped graphene frameworks, leading to the formation of advanced structures like $M-N_x-C$, M , $M'-N_x-C$, or $M-S$, N_x-C frameworks. These hybrid structures significantly enhance the catalytic architecture by further optimizing the redistribution of electronic density and modifying electron transfer pathways. A prominent example is the $Fe-N_4$ configuration, which remarkably mimics the active center (iron-heme group) of natural heme-containing peroxidases. This biomimetic structure exhibits profoundly enhanced peroxidase-like activity by effectively reducing the energy barrier for the generation of hydroxyl radicals. Similarly, $Zn-N_x$ -structured GO and $Cu-N_4S-C/Cu-N_4-C$ -structured GO demonstrate exceptional peroxidase-like activity. The integration of metal nanoparticles, such as Fe_3O_4 , onto the graphene oxide surface is also highly effective, preventing particle aggregation and thereby exposing a greater number of active sites, which leads to a significant enhancement in catalytic activity.

Recent advancements include single-atom nanozymes (SANs),⁹⁹ where graphene-based nanomaterials support atomically dispersed metal active sites. This integration maximizes atom utilization and enhances catalytic efficiency and stability compared to traditional enzyme mimetics. The synergy between SANs and graphene enables precise control over catalytic activity through modifications to the graphene structure and the selection of metal atoms. This tunability is essential for optimizing enzymatic functions across various applications, including sensing, environmental remediation, and biomedical uses.^{100,101} As research progresses, the ability to tailor the properties of SANs within graphene composites is expected to lead to breakthroughs in both fundamental research and practical applications, establishing them as a focal point in the development of next-generation nanomaterials.

The integration of machine learning techniques has further enhanced the tunability of graphene-based nanozymes by predicting and optimizing conditions for desired catalytic activities.¹⁰² This high degree of tunability has led to significant advancements in various fields, including biosensing, where these nanozymes can be used to develop sensitive and selective sensors for detecting biomolecules crucial in medical diagnostics and environmental monitoring. In clinical therapy, the enzyme-like activities of tunable graphene-based nanozymes can be harnessed for drug delivery systems, catalyzing reactions that release drugs at targeted sites within the body. Furthermore, their applications extend to environmental remediation, pollution control, and agricultural analysis, showcasing these tunable nanomaterials' versatility and potential impact across



multiple industries.^{65,103,104} As research in this field progresses, the tunability of graphene-based nanozymes promises to revolutionize various industrial and biomedical applications by providing customizable and efficient catalytic solutions.

4. Biomedical application of graphene-based nanozymes

Graphene-based nanozymes have emerged as promising candidates in biomedicine due to their unique properties and exceptional catalytic capabilities. These nanozymes, composed

of graphene,¹⁰⁵ graphene oxide,¹⁰⁶ reduced GO (rGO),^{107,108} and graphene quantum dots (GQDs)¹⁰⁹ loaded with various metallic nanoparticles, mimic the enzymatic functions of natural enzymes. Their exceptional biocompatibility, high surface area, and tunable surface chemistry make them ideal for biomedical applications. These nanozymes exhibit strong antioxidant properties, scavenging harmful reactive oxygen species and protecting cells from oxidative stress-induced damage.¹¹⁰ Moreover, their peroxidase-like activity enables the efficient detection and removal of toxins, pathogens, and pollutants in biological environments.¹¹¹ The biomedical application of graphene-based nanozymes holds great promise for advancing

Table 2 Overview of graphene-based nanocomposites: biomimetic activities and their applications across various fields^a

Nanocomposite	Enzymatic activity	Applications	References
CuS/GO NC	Oxidase and peroxidase	Antibacterial and wound healing	120
rGO-GP	Peroxidase	L-Cysteine detection	68
CoO/N-CS-rGO	Oxidase	Dopamine & uric acid detection	121
Au-rGO	Laccase	Phenolic substrate detection	122
Fe ₃ O ₄ @porous graphene	Peroxidase	Glucose detection	123
Ce-GONRs	Peroxidase and oxidase	Pesticides detection	106
rGO/MWCNT	Peroxidase	Glutathione detection	124
GQD-SPNs	Peroxidase	Photothermal cancer therapy	125
NPGQDs	Peroxidase	Chemodynamic cancer therapy	126
NH ₂ GQDs/ <i>o</i> -phenylenediamine	Oxidase	H ₂ O ₂ & ascorbic acid	127
N, B-LIGzyme	Peroxidase	Anti-bacterial	128
Fe-N-rGO	Peroxidase	H ₂ O ₂	58
PdNPs/N-PC-rGO	Oxidase, peroxidase, and catalase	Glutathione detection	129
Pt/NG	Peroxidase	Acetylcholinesterase sensing	130
Cu _{2-x} Se/rGO	Peroxidase	Cancer cell detection	131
CuONCs/rGO	Peroxidase	H ₂ O ₂ & ascorbic acid	132
rGO-CMCS-hemin/Pt@Pd NPs	Peroxidase	Golgi protein	133
PRTM-RhNC@rGO	Uricase	Uric acid & inhibition of urate crystal	134
Fe ₃ O ₄ -NH ₂ /GONRs	Peroxidase and catalase	Sildenafil	135
Ag ₂ S@GO	Oxidase	Hg(II) sensing	136
Fe ₃ O ₄ @CuO-GO	Peroxidase	Homocysteine	137
GO-DNA-PtNPs	Peroxidase	Hg(II) sensing	138
GOD-GO/MnO ₂	Peroxidase	Glucose detection in blood	139
Cu-NS-rGO	Peroxidase	Catalytic activity	140
Cys-GO	Peroxidase	Hg(II) sensing	141
PtCo@graphene	Oxidase	Antibacterial	142
rGO/MoS ₂ and Fe ₃ O ₄ NPs	Oxidase and peroxidase	Cancer biomarkers	143
BN-GDY	Peroxidase	Cancer therapy	144
GQD/CoSnO ₃	Peroxidase	Chemo-sono dynamic cancer therapy	145
Cu ₃ /ND@G	Oxidase	Antibacterial	146
GO/CuFeS _x NC	Peroxidase	Antibacterial	147
MoS ₂ /rGO	Oxidase, peroxidase and catalase	Antibacterial	148
Cu/GO SACs	Superoxide dismutase	Treatment of lung injury and anti-inflammatory	149
N-GNMs	Peroxidase	Tumor-specific treatment	150

^a CuS/GO NC – copper sulfide nanozymes anchored to graphene oxide nanosheets; rGO-GP – reduced graphene oxide with Ganoderma polysaccharide; CoO/N-CS-rGO – CoO nanoparticles/N-doped carbon sheets/reduced graphene oxide composites; Au-rGO – gold nanoparticle with reduced graphene oxide; Fe₃O₄@porous graphene – magnetite on porous graphene; Ce-GONRs – cerium nanoparticles with graphene oxide nanoribbons; rGO/MWCNT – reduced graphene oxide multiwalled carbon nanotubes; GQD-SPNs – graphene quantum dots/semiconducting polymer; NPGQDs – N and P codoped graphene quantum dots; NH₂GQDs/*o*-phenylenediamine – amino-functionalized graphene quantum dots and *o*-phenylenediamine; N, B-LIGzyme – laser induced N-and B-codoped graphene nanozymes; Fe-N-rGO – iron-N embedded on reduced graphene oxide; PdNPs/N-PC-rGO – PdNPs functionalized N-doped porous C-rGO; Pt/NG – Pt nanoparticle/N-doped graphene; Cu_{2-x}Se/rGO – Cu_{2-x}Se nanoparticles on graphene oxide hybrids; CuONCs/rGO – CuO nanocluster supported by reduced graphene oxide; rGO-CMCS-hemin/Pt@Pd NPs – reduced graphene oxide-carboxymethyl chitosan-hemin/platinum@palladium; PRTM-RhNC@rGO – arginine-rich peptide-rhodium nanocluster@reduced graphene oxide composite; Fe₃O₄-NH₂/GONRs – monodispersed spherical composite magnetite on graphene oxide; Ag₂S@GO – silver sulfide on graphene oxide; Fe₃O₄@CuO-GO – Fe₃O₄, CuO, and cucurbit[6]uril on graphene oxide; GO-DNA-PtNPs – graphene oxide@DNA with platinum nanoparticles; GOD-GO/MnO₂ – glucose oxidase-conjugated graphene oxide/MnO₂ nanozymes; BN-GDY – boron and nitrogen codoped graphdiyne; Cu₃/ND@G – Cu₃ cluster stabilized on defect-rich nanodiamond-graphene; Cu/GO SACs – copper@graphene oxide single atom catalyst; N-GNMs – nitrogen-doped graphene nanoparticles.



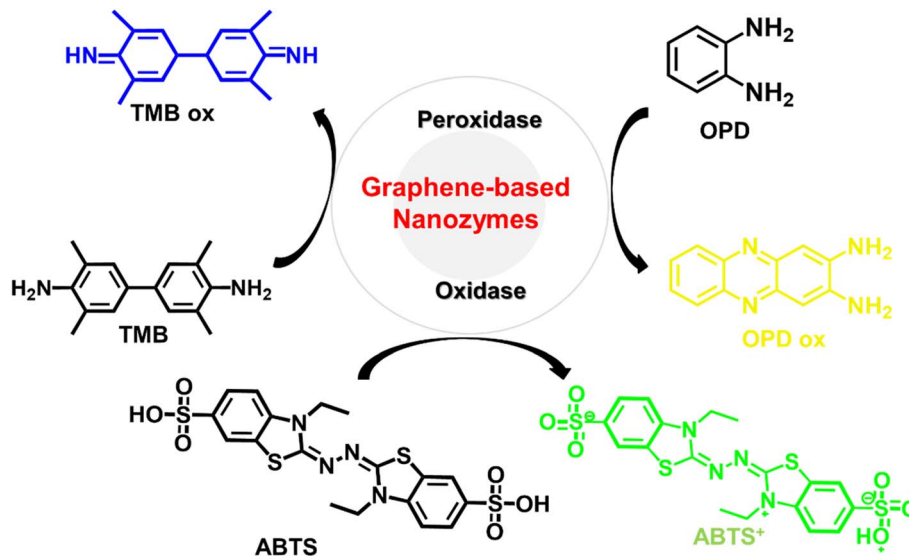


Fig. 6 Enzymatic activity of graphene-based nanozymes in biosensing.

disease diagnosis, therapy, and environmental protection, making them a cutting-edge technology to pursue improved healthcare and a healthier environment.

The nanozymes play a significant role in various biomedical applications. One key area is biosensing and diagnostics.¹¹² These nanozymes can be highly sensitive and selective platforms for detecting biomolecules and disease markers. By functionalizing graphene with specific receptors or antibodies, they can capture and detect target molecules with remarkable accuracy. This enables the early diagnosis of diseases such as cancer, infectious diseases, and cardiovascular disorders.

Graphene-based nanozymes also demonstrate outstanding potential in tissue engineering^{113,114} and regenerative medicine.^{115–117} Their biocompatibility, conductivity, and mechanical strength make them suitable scaffolds for promoting cell growth, proliferation, and differentiation.¹¹⁸ These nanozymes can be used to construct 3D tissue scaffolds¹¹³ that mimic the natural extracellular matrix, facilitating tissue regeneration and organ transplantation. Additionally, graphene-based nanozymes have been explored for their antibacterial properties.¹¹⁹ They can effectively inhibit bacterial growth and biofilm formation, making them valuable in combating antibiotic-resistant bacteria. Furthermore, these nanozymes can aid in wound healing by promoting tissue regeneration and preventing infections.⁴⁶ Overall, the role of graphene-based nanozymes in biomedical applications is vast and diverse, as discussed in Table 2. They offer tremendous potential in biosensing, drug delivery, tissue engineering, antibacterial treatments, and other areas, revolutionizing the field of biomedicine and improving patient outcomes.

4.1 Biosensing applications

Graphene nanozymes have revolutionized biosensing by combining the unique properties of graphene-based nanoparticles with enzymatic catalytic activity. These nanomaterials, designed to mimic natural enzymes, exhibit exceptional

catalytic efficiency and specificity, making them crucial for detecting biomolecules in various applications.^{53,151} Graphene-based nanomaterials as colorimetric¹⁵² and electrochemical biosensors,¹⁵³ provide unique properties for the *in situ* detection of glucose, DNA, enzymes, proteins, and microorganisms.^{154,155} They also show potential in cancer cell detection,¹⁵⁶ enabling early and accurate diagnosis. This activity allows the nanozymes to catalyze the oxidation of colorimetric substrates like TMB (3,3',5,5'-tetramethylbenzidine), and *ortho*-phenylenediamine in the presence of H₂O₂, resulting in a color change that can be detected colorimetrically (Fig. 6). Their combination with polymers and surface-decorated metal nanoparticles expands their potential in biosensing. Graphene-based nanozymes offer a versatile platform for efficient detection and advancements in biomedical research and diagnostics (Table 3).

For instance, carboxyl-modified graphene oxide (GO-COOH) possesses inherent sensing capabilities, enabling the detection of ions and small molecules.¹⁷⁴ This functionalization enhances the surface area and facilitates binding interactions, increasing the sensitivity of biosensors. Furthermore, the integration of gold nanoparticles with graphene, creating a composite known as Au-rGO, has led to the development of advanced biosensors.^{122,165} This hybrid material benefits not only from the catalytic properties of graphene but also from the unique properties of gold nanoparticles, making it a versatile platform for biosensing.^{8,175} In point-of-care diagnosis, graphene nanozymes show great promise. One notable example is the creation of glucose-biosensing microneedle patches. These patches consist of GOx-conjugated MnO₂/graphene oxide nanozymes (GOx-MnO₂@GO), enabling non-invasive and efficient glucose monitoring.¹³⁹ This innovation has the potential to revolutionize the management of diseases like diabetes, providing patients with convenient and accurate monitoring solutions.

The broader impact of graphene nanozymes is reflected in their diverse applications beyond biosensing. Their innate catalytic behavior, similar to biological enzymes, extends their



Table 3 List of nanozymes with their enzyme-like activities, analyte, method, and limit of detection (LOD)^a

Nanocomposite	Enzyme mimic	Detection	Method	LOD	References
NGQD@NC@Pd HNS	Catalase	H ₂ O ₂ , cancer cells	Electrochemical	20 nM	157
NiCo ₂ O ₄ /3DGF	Catalase	Glucose and calcium ion	Electrochemical	0.38 and 4.45 μM	158
GCNT-Fe ₃ O ₄	Peroxidase and catalase	H ₂ O ₂ and glucose	Colorimetric and electrochemical	0.022 mM	159
GSF@AuNPs	Peroxidase	Cancer cells and therapeutics	Colorimetric	—	45
NB-rGO	Peroxidase	Acetylcholine and C-reactive protein	Colorimetric	10 nM	160
3DRGO-Fe ₃ O ₄ -Pd	Peroxidase	Glutathione and glucose	Colorimetric	13 μM	161
RGO-IN	Peroxidase	H ₂ O ₂ and glucose	Colorimetric	0.2 and 0.8 μM	151
γ-Fe ₂ O ₃ /t-SG	Peroxidase	Chlorpyrifos	Colorimetric	0.17 μg mL ⁻¹	162
b-Fe-GQDs, o-GQDs	Peroxidase and oxidase	Alkaline phosphatase	Colorimetric and fluorescence	0.21 U L ⁻¹	163
Pt/NG	Peroxidase	Acetylcholinesterase	Colorimetric	0.0652 mU mL ⁻¹	164
AuAg-rGO	Peroxidase	Glutathione	Colorimetric	38 nM	165
Zr-GO	Peroxidase	H ₂ O ₂	Colorimetric	0.57 μM	166
CoO/N-CS-rGO	Oxidase	Dopamine and uric acid	Electrochemical	1378 μA mM ⁻¹ and 1393 μA mM ⁻¹	121
NH ₂ -MIL-88B(Fe)@MRGO	Peroxidase	Glucose	Colorimetric	3.16 μM	154
RuO ₂ /rGO	Peroxidase	Glucose	Colorimetric	3.34 μM	167
WS ₂ /rGO	Peroxidase	H ₂ O ₂	Colorimetric	82 nM	168
Co-His-GQD-G	Oxidase	Chlorpyrifos	Colorimetric	0.57 ng mL ⁻¹	169
FA-MnO ₂ /GO	Oxidase	Cancer cells	Colorimetric	20 cancer cells	170
g-CNQDs and GQDs	Peroxidase	Fluoride ion	Colorimetric and fluorescent	4.06 μM	111
rGO/CMCNs	Peroxidase	Dopamine in blood serum and urine samples	Colorimetric	0.17 μM	171
CGNPs	Peroxidase	Dopamine	Colorimetric	1.15 μM	62
Fe ³⁺ -NGQDs	Peroxidase	Hydroquinone	Colorimetric	0.2 μM	65
Fe, N-GQD	Peroxidase	L-Cysteine	Colorimetric	140 nM	66
GQDs and MoS ₂ QDs	Peroxidase	Cholesterol	Chemiluminescent	35 nM	172
PtCu/PSS-Gr	Peroxidase	Puerarin	Colorimetric	1 × 10 ⁻⁵ M	173

^a NGQD@NC@Pd HNS – Pd nanoparticles decorated N-doped graphene quantum dots@N-doped carbon hollow nanospheres; NiCo₂O₄/3DGF – nickel-cobalt oxide decorated three-dimensional graphene; GCNT-Fe₃O₄ – cubic Fe₃O₄ nanoparticles loaded on graphene oxide-dispersed carbon nanotubes; GSF@AuNPs – gold nanoparticle-loaded mesoporous silica-coated graphene; NB-rGO – N- and B-codoped reduced graphene oxide; 3DRGO-Fe₃O₄-Pd – three-dimensional graphene-magnetic palladium nanohybrids; RGO-IN – reduced graphene oxide-iron nanoparticles; γ-Fe₂O₃/t-SG – γ-Fe₂O₃/thiophene-sulfur-doped graphene; b-Fe-GQDs, o-GQDs – iron and carbonyl functionalized graphene quantum dots; Pt/NG – Pt nanoparticle/N-doped graphene; AuAg-rGO – reduced graphene oxide capped AuAg bimetallic nanoparticles; Zr-GO – zirconium on graphene oxide; CoO/N-CS-rGO – CoO nanoparticles/N-doped carbon sheets/reduced graphene oxide composites; NH₂-MIL-88B(Fe)@MRGO – NH₂-MIL-88B(Fe)@modified reduced graphene oxide; RuO₂/rGO – ruthenium oxide/reduced graphene oxide; WS₂/rGO – tungsten disulfide on reduced graphene oxide; Co-His-GQD-G – cobalt histidine graphene quantum dot on graphene; FA-MnO₂/GO – folic acid functionalized MnO₂ on graphene oxide; g-CNQDs and GQDs – fluorescent graphitic carbon nitride and graphene oxide quantum dots; rGO/CMCNs – reduced graphene oxide Cu₃(OH)₂(MoO₄)₂ cuboidal nanostructures; CGNPs – crumpled graphene nanoparticles; Fe³⁺-NGQDs – nitrogen-doped graphene quantum dots and iron ion nanocomposite; GQDs and MoS₂ QDs – graphene quantum dots and molybdenum disulfide quantum dots; PtCu/PSS-Gr – PtCu bimetallic nanoparticle on polystyrene sulfonate functionalized graphene.

use to other fields, such as drug delivery, environmental monitoring, and energy conversion. This versatility, coupled with their exceptional properties, positions graphene nanozymes as a cornerstone in the ongoing advancement of cutting-edge technologies for both diagnostics and therapeutics.^{176–178} For instance, manganese phthalocyanine-modified graphene nanosheets function as a peroxide-like nanozyme, catalyzing the oxidation of TMB by H₂O₂ (Fig. 7).

4.1.1 Detection of cancer cells. Cancer is a widespread and devastating disease that claims numerous lives globally. As a result, the timely diagnosis of cancer is of utmost

importance.¹⁷⁹ Detecting cancer at its earliest stages can significantly improve patients' prognosis and increase the effectiveness of treatment.¹⁸⁰ Given the high mortality rates associated with cancer, early detection holds the key to improving survival rates and reducing the impact of this disease on individuals and communities worldwide.¹⁸¹ Therefore, developing and utilizing accurate and sensitive diagnostic methods is essential in the ongoing battle against cancer. Early diagnosis not only enhances individual outcomes but also contributes to broader efforts to combat and mitigate the effects of cancer on a global scale.¹⁸² Cancer cell detection is a crucial



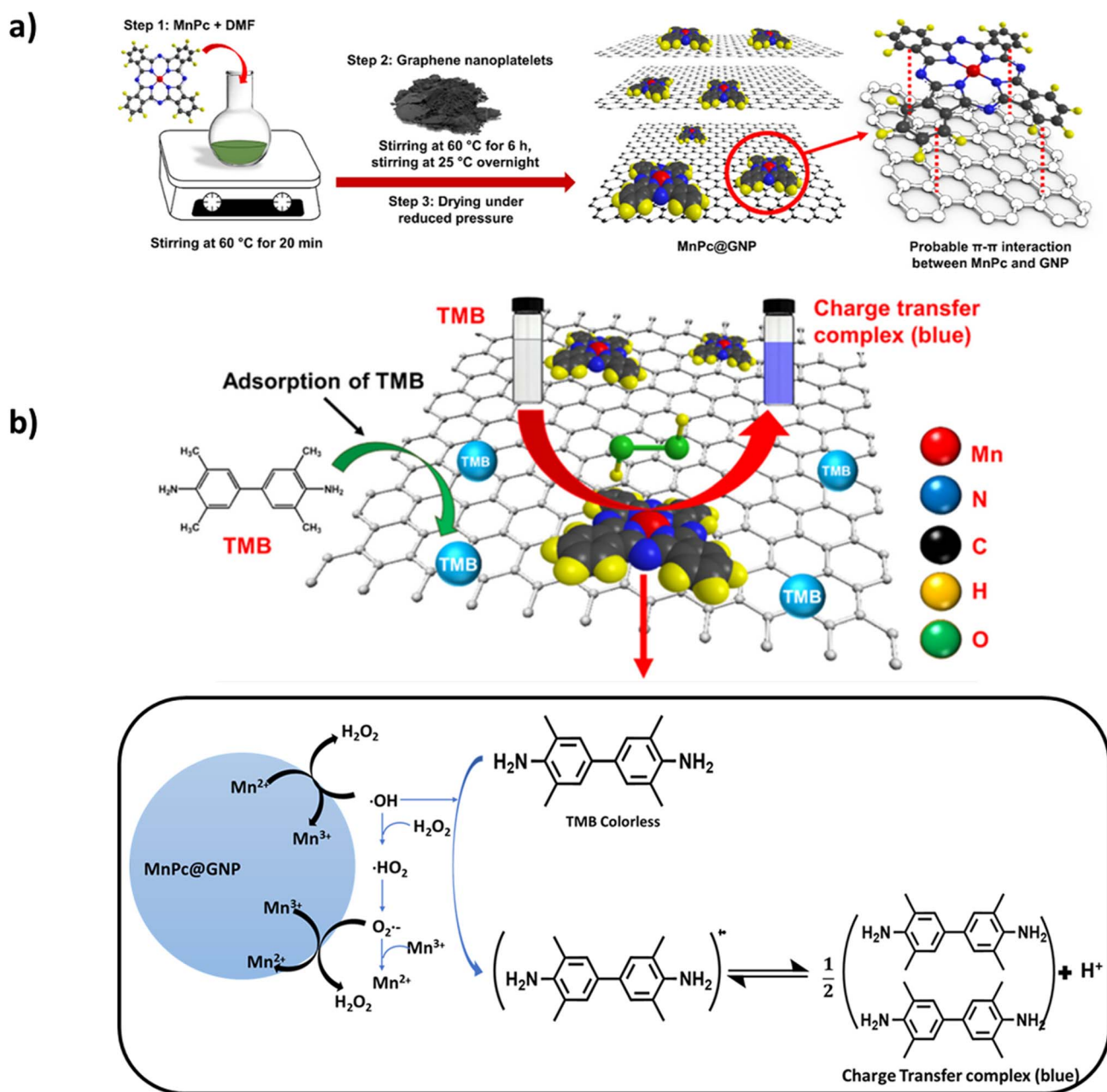


Fig. 7 (a) Diagrammatic representation for the synthesis of MnPc@GNP (manganese phthalocyanine on graphene nanosheet) (b) plausible mechanism for the H_2O_2 mediated TMB oxidation using MnPc@GNP. Reprinted with permission from ref. 176. Copyright 2023 American Chemical Society.

aspect of early cancer diagnosis and treatment. Detecting cancer cells at an early stage can significantly improve the chances of successful treatment and recovery for patients. Various methods and technologies, such as imaging techniques,¹⁸³ flow cytometry,¹⁸⁴ and immunophenotyping¹⁸⁵ are employed to identify and analyze cancer cells within the body. These detection methods aim to pinpoint abnormal cellular activity, genetic mutations, or the presence of specific biomarkers associated with cancer. Despite their established status, these approaches are expensive, time-consuming, and susceptible to operator variance. Therefore, there is a growing

requirement for alternative cancer cell detection methods that are swift, sensitive, and affordable.

Graphene-based nanozymes are good alternatives for the early detection of cancer cells. Liu *et al.* fabricated a MnO_2 nanosheet/graphene oxide hybrid functionalized with folic acid that served as an oxidase mimic.¹⁸⁶ According to these findings, FA- MnO_2/GO might preferentially bind to cancer cells by interacting with the folate receptors that are overexpressed in many tumor and cancer cell lines. Additionally, it catalyzed the oxidation of TMB without the use of H_2O_2 . The overexpression of the folate receptor on hybrid and cancer cells was made



possible by the functionalization of folic acid (FA), and the binding events were then transformed into quantifiable colorimetric signals by the oxidation of the chromogenic substrate TMB. In another study, Tao *et al.* discussed a coordinated strategy for cancerous cell detection, reporting the incorporation of AuNCs in graphene oxide-based colorimetric biosensors with amplified signals for cancer cell detection due to the favorable optical properties, biocompatibility, and low toxicity of AuNCs.¹⁸⁷ Good catalytic oxidation of TMB occurred even at neutral pH by incorporating AuNC in graphene oxide, enabling selective, sensitive cancer cells detection by leveraging the specific binding between folic acid conjugated to the graphene oxide and folate receptors on cell membranes, achieving a detection limit of 1000 MCF-7 cells. In another study, Kim *et al.* significantly improved the activity of a nanohybrid by modifying the surface of graphene oxide with platinum nanoparticles (PtNPs) and Fe₃O₄ magnetic nanoparticles.¹⁸⁸ This nanohybrid exhibited a 30 times higher maximal reaction velocity (V_{\max}) compared to bare graphene oxide and blue color signals obtained in just 5 minutes, proportional to the amount of target cancer cells.

Guo *et al.* utilized a nanocomposite of Cu_{2-x}Se/reduced graphene oxide, aided by folic acid, to visualize cancerous cells. To functionalize Cu_{2-x}Se/rGO, folic acid was chosen as the target chemical since it can efficiently target various tumor cells with overexpressed folate receptors on their cell membranes.¹³¹ FA-Cu_{2-x}Se/rGO was demonstrated to possess the capacity to distinguish between target cells and control cells. Furthermore, Zhang *et al.* designed the colorimetric test to quickly and accurately identify cancer cells using PtNPs/GO as a signal transducer. Folic acid was used as the recognition component to functionalize PtNPs/GO.¹⁸⁹ When the folate receptor on a tumor cell's membrane was overexpressed, folic acid efficiently targeted those cells. PtNPs/GO on tumor cells transformed the identification process into a quantifiable colorimetric signal, providing an accurate and convenient method for identifying malignant cells. Folic acid was conjugated with hybrid nanomaterials, resulting in the FA-GO-AuNCs composite (GFA), which served as an effective probe for the rapid, selective, and quantitative colorimetric detection of cancer cells. As reported by Yu Tao *et al.*, this technology could substantially improve diagnostic accuracy and prognostic monitoring in oncology.¹⁸⁷ To create a quick, low-cost, highly accurate, and sensitive colorimetric test to identify cancer cells, they used the folic acid-attached GO-AuNCs hybrid.¹⁸⁷

Electrochemical biosensors are being extensively used in fields such as clinical diagnostics, environmental monitoring, and food safety analysis, as they are user-friendly, cost-effective, highly stable, and offer excellent sensitivity.¹⁵⁵ Alizadeh *et al.* created a 3-electrode electrochemical cell, a cost-effective, rapid, and highly sensitive technique for cancer cell identification.¹⁵⁶ They discovered that the electrochemical detection of cancer cells required electrodes and a sensing platform. The CuO/WO₃ nanoparticles, which exhibit peroxidase-like activity, may be incorporated into this sensing platform. CuO/WO₃ nanoparticles catalytically converted H₂O₂ generated by cancer cells into ROS upon contact. This enzymatic activity enhanced the

electrochemical signal, making the presence of cancer cells more straightforward to detect. Liu *et al.* revealed that a graphene hemin composite coated with Au nanoflowers can exhibit enhanced peroxidase-like characteristics, making it a promising electrochemical aptamer biosensor for detecting K562 leukemia cells.¹⁹⁰ They also employed a three-electrode arrangement, with the gold electrode as the working electrode. Cancerous cells can be detected for several reasons. For example, the biosensor contains aptamers that are precisely chosen to recognize and bind to the target molecules on the surface of K562 leukemia cancer cells. When malignant cells are present in the sample, the aptamers bind specifically to them, enabling their recognition. The signal amplification process plays a significant role in their experiment. AuNFs inserted within the biosensor provide additional catalytic sites, enabling signal amplification. The catalytic capabilities of AuNFs increase the electrochemical process, resulting in an amplified signal that correlates to the presence or amount of K562 leukemia cancer cells. This amplification step improves detection sensitivity. Nanozymes capture electroactive molecules due to their large surface area and numerous binding sites, which enable more effective electrochemical reactions.

In a recent study, Song *et al.* introduced an innovative composite called folic acid-linked graphene-hemin (GFH).¹⁹¹ This compound demonstrated the ability to rapidly, selectively, and precisely target cancer cells *via* a colorimetric signal enhanced by the peroxidase-mimicking activity of GFH. This method can detect cancer cells at a threshold of 1000 cells or less. Compared to other methods that rely on antibodies or natural enzymes, this composite offers superior stability and resistance to denaturation or degradation. Additionally, graphene in this composite offers several advantages over other materials, such as carbon nanotubes or mesoporous silica. The significant size of the GFH complex allows for its visualization under an optical microscope, facilitating its entry into cells. The system is capable of detecting cancer cells within cellular environments by focusing on surface receptors or specific biomarkers. The strong π - π stacking interactions between graphene and hemin facilitate the attachment of multiple hemin molecules to both sides of a single graphene layer. This arrangement ensures that each hemin active site is easily accessible for interaction with substrates, significantly enhancing catalytic efficiency. Graphene oxide (GO) also possesses numerous functional groups, including epoxy, hydroxyl, and carboxyl, allowing biomolecules to attach flexibly. As a result, our system shows significant promise for a wide range of applications. The findings from this research could enhance the application of functionalized graphene derivatives in various fields, including accurate and rapid recognition of specific cells with outstanding sensitivity, DNA and protein analysis, and the development of new imaging methods.¹⁹¹ The GO possesses numerous functional groups, allowing biomolecules to attach readily. The system has potential applications in various fields, including sensitive cell detection, DNA and protein analysis, and advanced imaging techniques.

4.1.2 Hydrogen peroxide detection. Hydrogen peroxide (H₂O₂) is a crucial oxidizing agent generated during various



physiological processes. However, its potent oxidative activity poses a threat to cellular components, such as proteins and DNA. Detecting H_2O_2 has proven instrumental in studying disease progression, particularly for early-stage vascular diseases. An abnormal concentration level of H_2O_2 can lead to myocardial infarction, Alzheimer's disease, Parkinson's disease, cancer, *etc.*¹⁹² The graphene frameworks offer outstanding stability, expansive surface area, and superior electrical conductivity, providing a promising platform for enhanced catalytic properties. Several studies have demonstrated that these nanozymes exhibit remarkable efficiency in H_2O_2 detection, characterized by high sensitivity, specificity, and selectivity. Moreover, graphene-based nanozymes have been utilized to simultaneously detect H_2O_2 and other biomolecules by exploiting their catalytic properties.

The association between H_2O_2 and glucose is fascinating, as H_2O_2 is a byproduct of glucose oxidation mediated by glucose oxidase. Therefore, in clinical and basic research, it is common to employ compatible methodologies to detect H_2O_2 and glucose simultaneously. This parallel analysis enables a comprehensive investigation of the interplay between glucose

metabolism, oxidative stress, and pathological conditions, facilitating the development of diagnostic and therapeutic strategies. Zhang *et al.* designed a catalyst showing synergistically enhanced peroxidase-like activity by employing graphene with gold & platinum MOF (AuPt/MOF-graphene) using OPD.¹⁹³ They utilized it for the electrochemical detection of H_2O_2 , achieving a detection limit of 19 nM. Jin *et al.* fabricated a nanozyme showing good peroxidase activity using graphene oxide and gold to detect H_2O_2 using the electrochemical method with an estimated detection limit of 1.9 nM.¹⁹⁴ Furthermore, due to their vast active centers, Li *et al.* developed an MOF using the peroxidase activity of Fe_3O_4 , graphene, and $\text{NH}_2\text{-MIL-88B(Fe)}$. $\text{NH}_2\text{-MIL-88B(Fe)}@\text{MRGO}$ was utilized for glucose detection.¹⁵⁴ This mimic enzyme exhibited a high catalytic velocity ($2.57 \times 10^{-7} \text{ M s}^{-1}$) and affinity ($K_m = 0.0091 \text{ mM}$) for H_2O_2 substrates. The limit of detection (LOD) for glucose was found to be $3.16 \mu\text{M}$.

4.1.3 Detection of small molecules of biological importance. Small molecules include chemicals and biochemical agents, such as cholesterol, dopamine, and uric acid. Detecting these agents is helpful as they are the key biomarkers of disease.

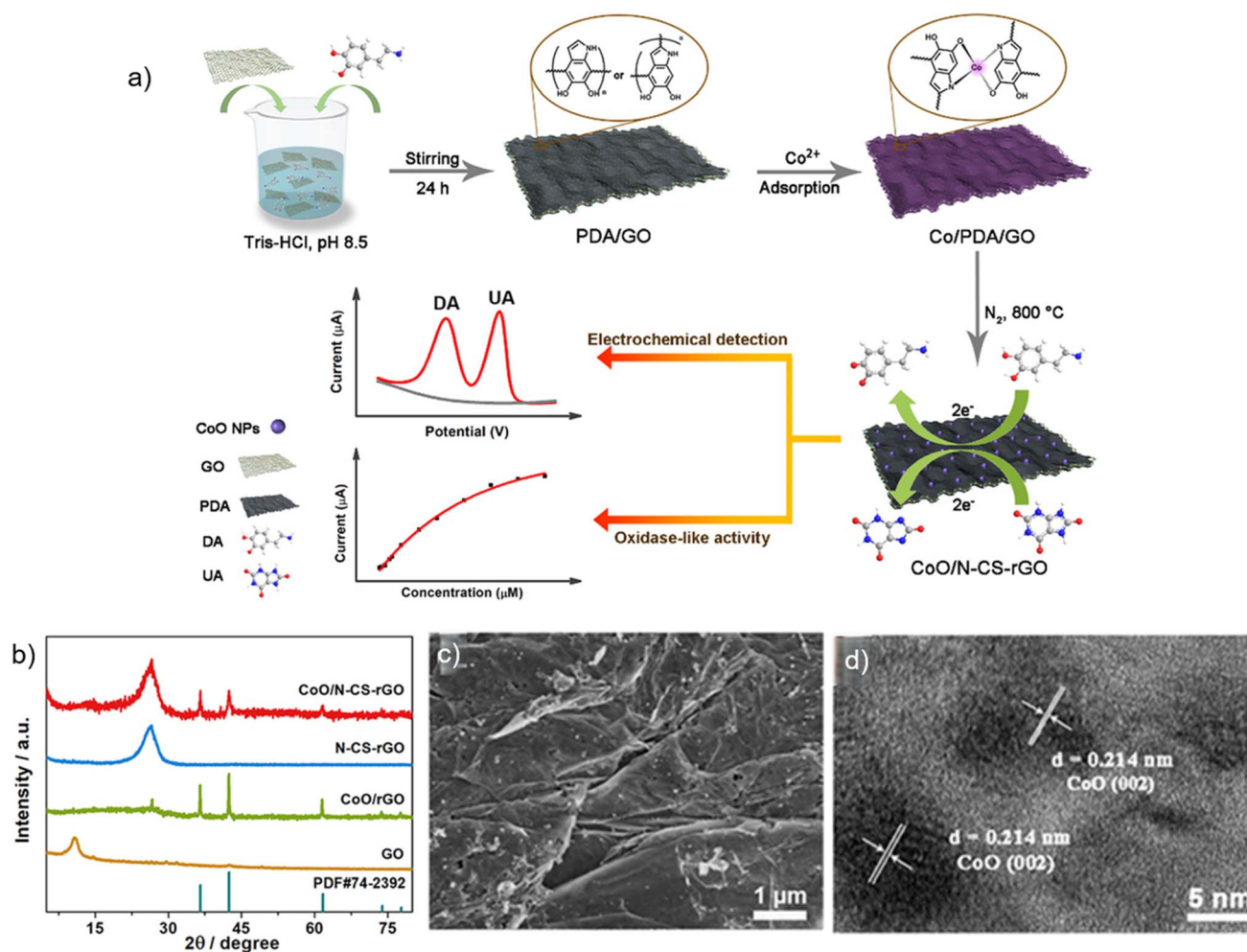


Fig. 8 (a) Schematic illustration of the synthesis of CoO/N-CS-rGO and sensing of dopamine and uric acid (b) XRD (c) SEM and (d) TEM images of CoO/N-CS-rGO. Reprinted with permission from ref. 121. Copyright 2022 American Chemical Society.



The functionalization of graphene-based nanozymes with specific receptors or ligands enables them to recognize and bind to target small molecules selectively. Upon interaction with the target molecule, the nanozymes catalyze a reaction that produces a discernible signal, such as fluorescence response, color change, or electrochemical response. As dopamine and uric acid are interlinked and produce similar signals, it is easy to detect them simultaneously. Using the hydrothermal method, Boruah *et al.* prepared 3D nitrogen-doped crumpled graphene nanoparticles (CGNPs).⁶² They used it to detect dopamine (DA) with high sensitivity in complex biological media, such as blood serum. Lu *et al.* synthesized a composite material consisting of polydopamine surface-functionalized cobalt oxide nanoparticles, N-doped carbon sheets, and reduced graphene oxide (CoO/N-CS-rGO) through an adsorption process followed by pyrolysis (Fig. 8). This composite exhibited oxidase-like activity. It was employed for the electrochemical detection of dopamine and uric acid. Structural characterization confirmed the presence of cobalt oxide, N-doped carbon sheets, and rGO, as indicated by XRD analysis, which revealed their characteristic peaks. SEM and TEM images further demonstrated that the N-doped carbon sheets are well-dispersed on the rGO surface, resulting in a distinctive sandwich-like morphology, as illustrated in Fig. 6.¹²¹ Novel graphene-supported ferric porphyrin and streptavidin were also investigated for their role as a peroxidase mimic for DNA.¹⁵⁵ This method combines probe-conjugated gold nanoparticles with graphene-coated electrodes and utilizes enzyme-functionalized carbon spheres as tracers. Combining these materials enables triplex signal amplification, enhancing the sensitivity and detection limit to the attomolar level in electrochemical DNA biosensing.

Furthermore, graphene-palladium nanowires have been utilized as an effective peroxidase mimic for electrochemical sensing. By incorporating ZnFe₂O₄-graphene quantum dots, the nanowires exhibit peroxidase-like activity, enabling the detection of small molecules. Glutathione (GSH), considered an essential antioxidant in biological systems, can be detected using a hemin dispersion by graphene quantum dots.¹⁹⁵ The nanozyme-based sensor shows a linear response to GSH concentrations ranging from 0.1 to 100 μM, with a detection limit of 0.01 μM.

Ascorbic acid (AA) functions as a cofactor in various physiological and metabolic processes. Inadequate or excessive AA can cause scurvy and anaemia, as well as different psychological disorders. As a result, it is crucial to develop a straightforward and rapid method with superior selectivity and precision for identifying AA in physiological and clinical analyses.¹⁹⁶ In recent years, several technologies for detecting AA have been developed, including fluorescence,^{184,185} ultra-performance liquid chromatography (UPLC) with a UV detector,¹⁸⁷ and chemiluminescence.¹⁹⁷ However, the nanozymes-based approach is more effective and simpler. AA may modify the peroxidase-like activity of nanomaterials, resulting in a color shift that can be visually observed. Based on the antioxidant characteristics of AA, peroxidase-based nanoprobe were reported to detect AA with reasonable accuracy. Sun *et al.* performed the colorimetric

detection of AA using the IrO₂/GO-H₂O₂-TMB reaction system.¹⁹⁸ The limit of detection (LOD) for ascorbic acid (AA) was determined to be 5.3 M. The colorimetric AA detection test was also done in simulated serum, vitamin C pills, and Mizone beverages to examine the practicality of the described approach for detecting AA in actual samples. Dong Peng *et al.* used a synthesis method to integrate reduced graphene oxide nano-sheets (p-rGO) with Mn₃O₄ nanoparticles (Mn₃O₄@p-rGO).¹⁹⁹ In this process, Mn²⁺ ions were adsorbed onto GO as a precursor, and a porous p-rGO structure was produced by directly annealing in open air. Decorating the surface of p-rGO nano-sheets with Mn₃O₄ nanoparticles significantly enhanced their oxidase-like activity, primarily due to the increased surface area, a higher density of active sites, and accelerated electron transfer efficiency. Furthermore, actual use for AA detection in real-world samples confirmed its practicality and dependability. This study demonstrates that decorating nanoparticles on specific supports is an effective technique for enhancing their enzyme-like functions, offering promising uses in pharmacy, food safety, biosensing, and clinical diagnosis.

4.1.4 Pesticide detection. Pesticides are chemical substances that are used to enhance plant growth and increase the supply of plant products. The use of pesticides leads to residues, which appear in processed commodities and eventually in the food chain. Boruah *et al.* synthesized Fe₃O₄/polydopamine functionalized graphene nanocomposites, which were established as nanozymes for detecting and degrading the pesticide simazine.²⁰⁰ The LOD (limit of detection) value for simazine was found to be 2.24 μM, and the nanozymes achieved 99% degradation of simazine. In another study, graphene oxide was used as an intrinsic peroxidase mimic for sensitively detecting organophosphate pesticides (OPs).²⁰¹ The colorimetric assay produces a measurable signal when AChE and ChO are present. OPs lead to the inhibition of AChE, resulting in a corresponding decrease in color intensity, which is directly proportional to the concentration of OPs. It was also reported that small-sized GO showed superior catalytic activity and higher stability and was used for OP detection. The corresponding LODs for dimethoate, methyl parathion, and chlorpyrifos were all found to be lower than 2 ppb, which is below the MRL, indicating their great potential for the quantitative analysis of pesticide residues. In another groundbreaking study, Boruah *et al.* introduce a dual-responsive magnetic Fe₃O₄-TiO₂/graphene nanocomposite as an enzyme mimic for both colorimetric pesticide detection and efficient photodegradation.²⁰⁰ The nanocomposite exhibits a synergistic enhancement in catalytic activity, yielding an impressive limit of detection (LOD) of 2.98 μg L⁻¹ for the detection of atrazine. Moreover, it effectively photodegrades atrazine into harmless compounds, contributing to environmental safety. The magnetic properties of these nanocomposites enable easy separation, simplifying the isolation process after degradation. This magnetic separability further facilitates the reuse of nanocomposites, enabling recyclability up to 10 times.

In another study, Tai *et al.* prepared cerium-doped graphene oxide nanoribbons (Ce-GONRs) with a three-dimensional porous structure evincing both peroxidase and oxidase like



activities, enabling synergistic oxidation of TMB.¹⁰⁶ This study revealed that diafenthuron and carbaryl pesticides inhibit the peroxidase and oxidase activity of Ce-GONRs, attributed to π - π stacking and hydrogen bonding between the pesticides and nanozymes. The detection limits were calculated to be 0.57 and 0.23 ng mL⁻¹ for diafenthuron and carbaryl, respectively. The detection was selective for these pesticides, whereas Lin *et al.* used the same nanocomposite for the detection of chlorpyrifos by inhibiting AChE, with a detection limit of 3.34 ng mL⁻¹ (0.0034 ppb).²⁰² Furthermore, another study demonstrates the synthesis of Co-histidine functionalized graphene quantum dot-graphene nanohybrid.¹⁶⁹ It evinced outstanding oxidase-like activity, superparamagnetic behaviour, and high stability. The method was applied to detect chlorpyrifos in peaches by inhibiting AChE. The detection limit was determined to be 0.57 μ g L⁻¹.

4.1.5 Detection of heavy metal ions. Due to their toxic and nonbiodegradable properties, as well as their tendency to accumulate in ecological systems, heavy metals pose significant challenges as pollutants. Recognizing the crucial role of monitoring environmental quality amid rising pollution from industrial, agricultural, and domestic sources, it is imperative to determine the presence of heavy metals accurately. This is especially essential given their persistence and potential harm to ecosystems. Consequently, the assessment of heavy metal content assumes immense significance in maintaining ecological balance and safeguarding ecosystem health.²⁰³ Graphene-based nanozymes play an important role in the detection of heavy metals. Song *et al.* developed a ternary Ag-CoFe₂O₄/rGO material using a microwave-assisted method.²⁰⁴ The resulting nanocomposites displayed impressive oxidase-like activity in catalyzing the oxidation of colorless TMB to blue oxTMB, as shown in Fig. 4. The incorporation of AgNPs into the nanocomposites significantly accelerated the formation of the Ag-Hg alloy in the presence of Hg²⁺. A strong synergistic effect was observed among AgNPs, the large surface area of CoFe₂O₄, and rGO, leading to the expedited decomposition of dissolved O₂ under weakly acidic conditions. As a result, in an acidic solution, O₂ adsorbed onto Ag-CoFe₂O₄/rGO nanocomposites could be readily converted into O₂⁻, which oxidized TMB to blue oxTMB. Leveraging the enhanced oxidase-like activity of Ag-CoFe₂O₄/rGO in the presence of Hg²⁺, a simple nanoenzyme-based sensor was developed for the colorimetric detection of Hg²⁺. The sensor exhibited a good linear range from 2 × 10⁻⁹ M to 1 × 10⁻⁸ M for Hg²⁺, with a low limit of detection (LOD) of 0.67 nM. Chen *et al.*, fabricated a GO-AuNP-based colorimetric sensor for the colorimetric detection of Hg²⁺ and Pb²⁺ simultaneously.²⁰⁵ This hybrid was further modified by introducing ssDNA to the GO-AuNP solution to form DNA-GO-AuNP, which exhibits enzyme-like catalytic activity and a salt aggregation effect. In the absence of Hg²⁺ or Pb²⁺, the hybrids remained stable even after the addition of NaCl. However, in the presence of Hg²⁺ or Pb²⁺, the structure of the hybrid changed, leading to the aggregation of DNA-GO-AuNP hybrids and the loss of their peroxidase-like activity at high NaCl concentrations. This resulted in the inability to catalyze the oxidation of TMB to oxTMB in the presence of H₂O₂. This unique phenomenon

enabled the development of an efficient label-free nanoenzyme-based colorimetric sensor with limits of detection (LODs) of 300 nM for Hg²⁺ and 500 nM for Pb²⁺.

In another study, Tao *et al.* reported that a colorimetric aptamer-based method for detecting Pb²⁺ was developed and validated using graphene/Fe₃O₄-AuNP composites as enzyme mimetics.²⁰⁶ The Pb²⁺ aptamer was anchored on magnetic beads functionalized with amine, and the presence of lead ions inhibited the catalytic activity and color reaction of the nanozyme composites. The method detected Pb²⁺ in the 1–300 ng mL⁻¹ range with a low detection limit of 0.63 ng mL⁻¹, showing potential for practical on-site analysis. This innovative approach has implications for addressing lead contamination and represents a significant advancement in colorimetric aptamer-based sensing.

4.2 Antimicrobial applications

Bacterial and microbial infections are on the rise, and there is a pressing need for antibacterial and antimicrobial agents to protect living organisms from disease-causing microorganisms. The common methods for combating bacterial infections primarily involve antibiotics, functionalized polymers,²⁰⁷ metal nanoparticles,²⁰⁸ metal oxide/sulfide nanostructures,¹¹ antibacterial peptides and antibacterial enzymes.²⁰⁹ While these approaches have generally been effective in preventing drug resistance, they still have limitations. For example, they may not be effective in combating infections caused by multidrug-resistant bacteria. Additionally, the simultaneous use of different antibiotics has led to increased antibiotic resistance, particularly in Gram-negative bacteria like *Escherichia coli* (*E. coli*), which can impede antibiotic entry into the cell and modify the cell wall to evade recognition by the antibiotic.²¹⁰

Graphene-based nanozymes have shown promising potential and catalytic behavior as the physical ability of graphene and its derivatives, such as their sharp-edge structure, to rupture bacterial membranes and their chemical properties, such as the production of oxidative stresses that deactivate bacterial proteins and lipids and disrupt the proliferation process, are the leading causes of their antimicrobial activity. Graphene-based nanozymes have unique catalytic properties that effectively kill bacteria by generating hydroxyl radicals, as summarized in Table 4. By using a simple hydrothermal technique, Wang *et al.* developed copper sulfide nanozymes attached to graphene nanosheets (CuS/GO NC), which exhibited potent oxidase and peroxidase-like activity.¹²⁰ CuS/GO NC exhibited physical and chemical activity against methicillin-resistant *Staphylococcus aureus* bacteria. Due to the needle-like morphology of CuS/GO NC, they punctured bacterial membranes, causing leakage of the intracellular matrix that rendered the bacteria inactive. They also caused damage to the bacterial cell by producing large amounts of ROS, leading to oxidative stress. In another study, hemin-reduced graphene oxide doped with gold nanoflakes (hemin-rGO@Au NFs) was made by Liu *et al.* and exhibited vigorous peroxidase-like catalytic activity, outstanding photothermal conversion efficiency in the NIR range, and quick electron transfer capability, due to



Table 4 Some graphene-based nanozymes for antibacterial applications^a

Nanoparticle	Enzymatic activity	Bacterial types and application	Antibacterial mechanism	References
GQDs	Peroxidase	<i>E. coli</i> , <i>S. aureus</i>	Generation of OH [•] radical	46
Au/g-C ₃ N ₄	Peroxidase	<i>E. coli</i> , <i>S. aureus</i>	Generation of OH [•] radical	47
GQD/AgNP hybrids	Peroxidase and oxidase	<i>E. coli</i> , <i>S. aureus</i>	Generation of OH [•] radical	187
PtCo@G	Oxidase	<i>H. pylori</i>	Generation of OH [•] radical	212
GQD/TiO _{2-x}	Peroxidase	<i>S. aureus</i> , <i>E. coli</i> , MRSA	Sonodynamic nano catalytic therapy	213
MoS ₂ /rGO VHS	Peroxidase, oxidase and catalase	<i>E. coli</i> and <i>S. aureus</i>	Generation of OH [•] radical	148
CuS/GO NC	Oxidase and peroxidase	MRSA	Generation of OH [•] radical	120
CoPt@G@GOx	Peroxidase	<i>Streptococcus mutans</i> biofilms	Generation of toxic OH [•] radicals in acidic environment	214
GO@Ag-Pt loaded nanozyme hydrogels	Superoxide dismutase	<i>S. aureus</i> , <i>E. coli</i> , wound healing	Antioxidant and ROS scavenger	48

^a GQDs – graphene quantum dots; Au/g-C₃N₄ – gold nanoparticle with graphitic carbon nitride; GQD/AgNP – graphene quantum dots and gold nanoparticles; PtCo@G – platinum and cobalt@graphene; GQD/TiO₂ – graphene quantum dots-sensitized TiO₂; MoS₂/rGO VHS – molybdenum disulfide/rGO vertical heterostructures; CuS/GO NC – copper sulfide anchored to graphene oxide nanosheets; CoPt@G@GOx – CoPt@graphene@glucose oxidase; GO@Ag-Pt – graphene oxide loaded silver-platinum hybrid nanoparticles.

which it showed synergistic antibacterial activity to Gram-negative drug-resistant bacteria (*E. coli*).²¹¹ Model bacteria, drug-resistant Gram-negative *E. coli*, were subjected to a solution containing the nanozyme composite (hemin-rGO@Au NFs) and a small amount of H₂O₂, which caused the generation of singlet oxygen (¹O₂), a reactive oxygen species with potent antibacterial properties. The singlet oxygen exhibited remarkable antibacterial activity following further NIR irradiation treatment, leveraging the composite's high photothermal conversion efficiency in the NIR range.

Another study investigated the antibacterial properties of graphene quantum dots (GQDs) and their potential to enhance the efficacy of hydrogen peroxide (H₂O₂) in combating bacterial infections.⁴⁶ GQDs exhibited superior enzymatic activity compared to GO due to their excellent electron transport properties, which enabled them to convert H₂O₂ into highly reactive hydroxyl radicals (OH), making them significantly more effective at killing bacteria. Importantly, GQDs demonstrated no significant toxicity in both *in vitro* and *in vivo*, making them a safer alternative for antibacterial applications. The combination of GQDs with low concentrations of H₂O₂ resulted in over 90% lethality against *Escherichia coli* and *Staphylococcus aureus*,

using H₂O₂ concentrations 100 times lower than those required when used alone. The mechanism of action involved the adherence of GQDs to bacterial membranes, facilitating the localized generation of OH radicals, which natural bacterial enzymes cannot easily neutralize. Additionally, the research led to the development of GQD-Band-Aids, which effectively prevented wound infections in mice; after 72 hours of treatment with these Band-Aids combined with H₂O₂, treated wounds showed no signs of erythema or edema (Fig. 9).⁴⁶ Overall, this work highlighted the potential of GQDs as a biocompatible and effective antibacterial agent, particularly in wound care applications where traditional antibiotics might be ineffective due to the development of resistance.

4.3 Application in wound healing

Graphene-based nanozymes have shown promising results in wound healing due to their excellent biocompatibility, catalytically active sites, mechanical strength, and electrical conductivity, making them suitable for biomedical applications. The antibacterial properties of graphene-based materials stem from their ability to disrupt bacterial cell membranes, resulting in

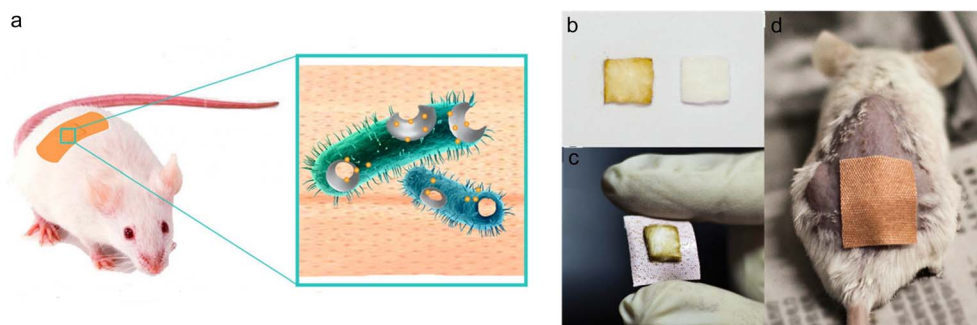


Fig. 9 (a) Schematic illustration of the GQD-assisted antibacterial system. (b) Picture of fabric absorbed GQDs. (c) The obtained GQD-Band-Aid. (d) The mouse treated with GQD-Band-Aid. Reprinted with permission from ref. 46. Copyright 2014 American Chemical Society.



cell lysis and improved wound healing outcomes. These materials can effectively inhibit the growth of pathogens, eliminate biofilm formations, and promote processes such as cell differentiation, re-epithelialization, and collagen deposition, all of which contribute to enhanced wound healing. For instance, graphene oxide nanoparticles (GONPs) have been found to significantly improve wound healing in animals, reaching up to 99% healed animals within 15 days.²¹⁵ Additionally, graphene-based nanozymes, such as Fe₃C/N-doped graphitic carbon, have shown efficient peroxidase-like activity, enabling broad-spectrum antimicrobial effects and accelerated wound healing *in vivo* by decomposing H₂O₂ to generate hydroxyl radicals, enhancing their antimicrobial efficacy.²¹⁶

The combination of graphene-based materials with other biocompatible components, such as polysaccharides and biological scaffolds, further improves their wound-healing potential. Chen *et al.* developed a multifunctional hydrogel by cross-linking polyvinyl alcohol and sodium alginate with GO that is loaded with silver–platinum hybrid nanoparticles (GO@Ag–Pt).⁴⁸ This advanced hydrogel exhibited remarkable abilities to scavenge ROS, produce oxygen, and demonstrate antibacterial activity against various bacteria *in vitro*. Adding silver–platinum hybrid nanoparticles significantly enhanced the hydrogel's antibacterial effectiveness against *Escherichia coli* and *Staphylococcus aureus* compared to conventional silver nanoparticles. Moreover, the GO@Ag–Pt loaded hydrogel successfully treated *S. aureus* infections, notably accelerating wound healing during the inflammatory phase by lowering ROS levels and reducing inflammation. This research highlighted the potential of ROS-scavenging and antibacterial hydrogels as viable treatments for various wound types, including challenging diabetic wounds complicated by bacterial infections, suggesting a promising approach for chronic wound healing through effective ROS management and bacteriostatic properties.⁴⁸

In recent years, antimicrobial resistance (AMR) has emerged as a significant threat to global health, necessitating the development of novel antibacterial agents. A study introduced a new nanozyme platform, Cu, N-GQDs@Ru–NO, which combined copper and nitrogen-doped graphene quantum dots (Cu, N-GQDs) with nitric oxide (NO) and ruthenium nitrosyl (Ru–NO).²¹⁷ When exposed to 808 nm near-infrared (NIR) light, this novel nanozyme exhibited remarkable NADH dehydrogenase-like activity, efficiently photo-oxidizing NADH to NAD⁺, thereby disrupting the redox balance within bacterial cells and ultimately leading to bacterial death. In addition to effectively eradicating methicillin-resistant *Staphylococcus aureus* (MRSA) and its associated biofilms, the NIR light also activated the NO donor, which facilitated wound healing. Excellent photothermal effects were another feature of the nanozyme, which enhanced its antibacterial effectiveness. By combining photothermal treatment, NO gas therapy, and NADH dehydrogenase activity, Cu, N-GQDs@Ru–NO exhibited remarkable *in vitro* and *in vivo* efficacy against bacterial resistant infections and biofilm eradication. This research presents a promising therapeutic strategy for treating MRSA-related inflammatory wounds through a multifaceted approach that targets bacterial survival and wound healing. This study aligns

with recent advancements in nanozyme research, where various metal-based nanozymes have demonstrated promise in combating antimicrobial resistance (AMR). For instance, other studies have explored the use of metal-free nanozymes, such as graphene quantum dots, for similar applications in cancer therapy and the management of oxidative stress. The unique combination of photothermal therapy and NO gas delivery distinguishes it from traditional therapies that may rely solely on one mechanism.²¹⁷ Additionally, integrating multiple therapeutic strategies into a single platform represents a significant advancement over conventional antibiotic treatments that often face challenges due to resistance mechanisms. Overall, this innovative approach highlights the potential of nanozymes as versatile tools in combating antimicrobial resistance (AMR) infections and promoting wound healing and tissue regeneration. Graphene-based nanozymes represent a frontier in wound healing technology, combining antimicrobial action with enhanced cellular proliferation and tissue regeneration capabilities. Continued exploration into their mechanisms and applications will likely yield innovative solutions for effective wound management.

4.4 Utilization as antioxidants

Graphene-based nanozymes have garnered considerable interest due to their exceptional antioxidant properties, making them promising candidates for addressing oxidative stress-related diseases, including neurodegenerative disorders, cardiovascular diseases, and cancer. These nanomaterials, particularly graphene oxide and reduced graphene oxide (rGO), exhibit significant free radical scavenging capabilities, effectively neutralizing ROS, such as hydroxyl radicals and hydrogen peroxide (H₂O₂). Research indicates that GO can outperform traditional antioxidants in protecting biomolecules from oxidative damage, showcasing its potential as a potent hydroxyl radical scavenger. The antioxidant efficacy of graphene materials is attributed to their unique structural characteristics, including high surface area and sp² carbon domains that facilitate electron transfer and radical adduct formation. Studies on graphene oxide quantum dots (GOQDs) have revealed their ability to significantly reduce ROS levels in neurotoxic environments, thereby preserving neuronal integrity.²¹⁸ A novel and highly efficient graphene oxide–selenium (GO–Se) nanozyme with outstanding antioxidative properties, designed to protect cellular components from oxidative damage.²¹⁹ This hybrid nanomaterial exhibited unique glutathione peroxidase (GPx)-like activity, effectively catalyzing the decomposition of hydrogen peroxide (H₂O₂) into harmless byproducts. Cell-based experiments confirmed its exceptional capacity to scavenge ROS, demonstrating its potential as a powerful antioxidant. These findings suggest that the GO–Se nanozyme could broaden the exploration of innovative selenium-based nanozymes.²¹⁹ Overall, the multifunctional nature of graphene-based nanozymes positions them as valuable tools in the development of antioxidant therapies to mitigate oxidative stress across various medical applications.



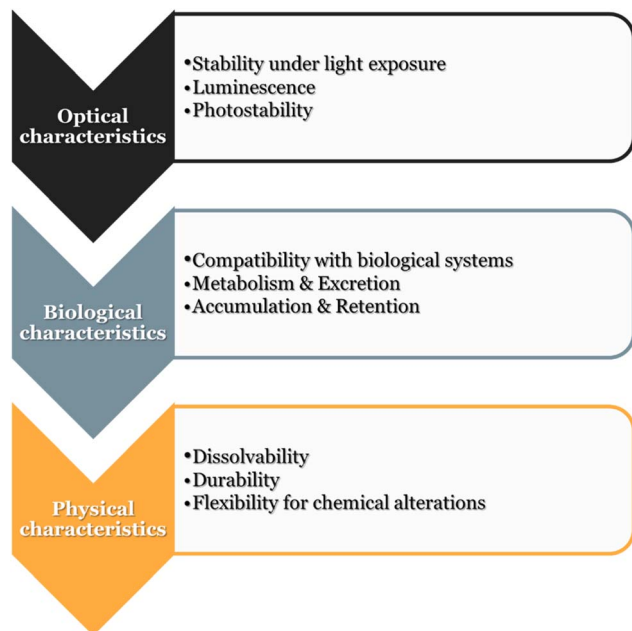


Fig. 10 Properties of graphene-based nanozymes useful in catalytic cancer therapy.

In-depth research is essential to optimize their design and fully understand their interactions within biological systems.

4.5 Graphene nanozymes in catalytic therapy

Catalytic therapy is a promising strategy for treating various diseases, including cancer and Alzheimer's, by using catalytic agents to trigger chemical reactions that either destroy cancer cells or break down toxic proteins associated with Alzheimer's disease. Because of their unique physicochemical properties, graphene-based nanozymes are particularly attractive as catalytic agents. They have high surface area-to-volume ratios, high electrical conductivity, and are highly biocompatible. These properties make them excellent candidates for use in catalytic therapy.

Graphene-based nanozymes possess unique properties that make them excellent candidates for targeted cancer treatment (Fig. 10).²²⁰ For instance, their high surface area enables optimal drug loading and delivery, while their catalytic activity facilitates the generation of ROS through oxidase and peroxidase activities. This ROS generation can selectively target and destroy cancer cells, offering a potential solution for the eradication of tumors. Furthermore, graphene-based nanozymes exhibit exceptional biocompatibility and can be easily functionalized to enhance their targeting capabilities.²²¹ They have demonstrated outstanding potential in overcoming limitations associated with traditional cancer therapies, such as drug resistance and off-target effects. Additionally, these nanozymes can be combined with other therapeutic strategies, such as chemophotothermal therapy, immunotherapy, and chemodynamic therapy, to enhance their efficacy further. These nanozymes have been the subject of extensive research owing to their intrinsic anticancer properties. Notably, they have

demonstrated the ability to improve cell adhesion, capture cancer cells, and induce tumor cell death through an oxidative stress mechanism.

Furthermore, studies have elucidated that graphene-based nanomaterials can reduce macrophage activity, thereby inhibiting cancer cell migration and invasion, ultimately suppressing tumor growth and metastasis.²²² Moreover, the versatility of these nanomaterials extends to their application in diverse modalities, such as drug delivery, gene therapy, phototherapy, and magnetothermal therapy in the context of breast cancer treatment. The multifaceted nature of graphene-based nanomaterials positions them as promising candidates for revolutionary cancer therapeutic strategies, leveraging their unique ability to regulate ROS and contribute to the development of advanced cancer therapies.

By harnessing the unique properties of graphene-based nanozymes, catalytic cancer therapy offers a promising avenue for targeted and effective treatment of tumors. The integration of graphene-based nanozymes in cancer therapy represents a significant advancement in the field, opening up new possibilities for more personalized and efficient treatment options with reduced side effects. The remarkable potential of these nanomaterials has sparked a wave of optimism within the scientific and medical communities, prompting further exploration and innovation in the realm of cancer treatment.²²³ As research continues to unveil the intricate mechanisms and capabilities of graphene-based nanomaterials, the prospects for groundbreaking advancements in cancer therapy appear increasingly promising. One notable application of graphene-based nanozymes is in photothermal therapy (PTT), which utilizes light-absorbing materials to convert light energy into heat, selectively destroying cancer cells (Fig. 11). Graphene quantum dots (GQDs) and graphene oxide quantum dots (GOQDs) are two types of graphene-based nanozymes that have recently attracted intense focus due to their high catalytic activity, similar to natural enzymes.²²⁴ These nanomaterials act as photothermal agents when exposed to near-infrared light, generating local heat and damaging cancer cells. The exceptional electrical, thermal, size-dependent optical, and magnetic properties of GQDs make them suitable for PTT applications, which can be further enhanced by incorporating them into nanocomposites.²²⁵

The potential of graphene-based nanozymes in PTT extends beyond their photothermal effect. These nanocomposites can also be utilized for drug loading and photodynamic impact, making them versatile tools in cancer treatment. Combining PTT with other therapies, such as chemotherapy or immunotherapy, holds great potential for improving cancer treatment outcomes. For instance, Hu *et al.* synthesized a graphene quantum dots/semiconducting polymer nanocomposite (GQD-SPN) that serves as both a temperature-sensitive nanozyme and a photothermal conversion agent.¹²⁵ GQD-SPNs, upon NIR laser irradiation, not only generate a massive concentration of $^1\text{O}_2$ but can also convert most of the optical energy into heat, thus raising the temperature within tumor regions locally. This enhanced photothermal effect further increases the peroxidase-like (POD) activity of the GQDs in the nanocomposite. The



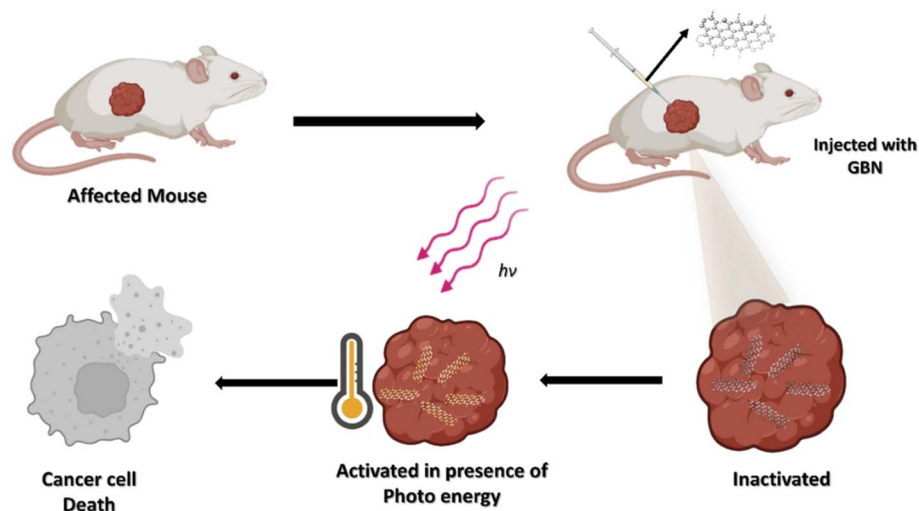


Fig. 11 Schematic representation of photothermal therapy in affected mouse model.

enhanced POD-like activity enables the GQD-SPNs to catalyze the endogenous hydrogen peroxide (H_2O_2) present in the tumor environment, leading to an abundance of hydroxyl radicals (OH). The synergistic action of the generated $^1\text{O}_2$, heat, and OH radicals produced by GQD-SPN nanocomposite under NIR irradiation resulted in enhanced outcomes in cancer therapy. This multifunctional nano platform further demonstrated the potential of rational graphene quantum dot-based nanomaterials for enhanced photodynamic and photothermal cancer treatment.¹²⁵

The photothermal effect generated by graphene-based nanozymes can exert a therapeutic effect on cells, thereby enhancing the effectiveness of anticancer agents. However, further research is needed to fully understand the *in vivo* behaviors of graphene-based nanomaterials and their long-term toxicology. In addition to PTT, graphene-based nanozymes have also shown promise in photodynamic therapy (PDT). This advanced two-stage therapeutic strategy utilizes light energy in combination with a drug (photosensitizer) to selectively eliminate cancer cells. The photosensitizer is nontoxic until it is activated by light.⁴⁹ Graphene-based nanozymes can be utilized as photosensitizers in PDT to generate ROS, such as singlet oxygen, which can induce the death of cancerous cells or pre-cancerous cells. Graphene nanozymes in PDT offer several advantages, including their excellent photothermal properties, large surface area for loading photosensitizers, and the ability to be easily functionalized for specific delivery to cancer cells.

In a study conducted by Maji *et al.*, a novel nanostructured hybrid, GSF@AuNPs, was synthesized to serve as a mimetic enzyme for *in vitro* diagnostic and therapeutic interventions in cancer cells.⁴⁵ The mixed material was created by attaching AuNPs to tiny pieces of reduced graphene oxide that had mesoporous silica on top. This nanozyme combines peroxidase-like activity and unique intrinsic properties of nanomaterials, making it suitable for imaging applications and the generation of toxic ROS under hypoxic conditions in the tumor microenvironment.⁴⁵ In another study by Alizadeh *et al.*, a CuO/WO_x -NPs decorated graphene oxide nanosheet was fabricated, showing

enhanced peroxidase activity.¹⁵⁶ The eradication of cancer cells has been achieved by generating OH^\cdot radicals from both externally supplied and internally generated H_2O_2 , facilitated by CuO/WO_3 -GO. Furthermore, Lin *et al.* synthesized graphene oxide nanoparticles (N-GO) by the chemical oxidation method, which augmented the ROS level in acidic pH conditions.²²⁶ In the acidic tumor microenvironment with high H_2O_2 levels, N-GOs catalyzed the oxidation of H_2O_2 into highly toxic hydroxyl radicals (HO^\cdot), resulting in the necrosis of tumor cells. Conversely, in a normal cell microenvironment with neutral pH, the N-GOs exhibit catalase-like activity, effectively scavenging ROS and causing no harm to normal cells. This selective behavior of N-GOs makes them highly promising for targeted action in the tumor microenvironment.²²⁶ Another study focusing on ferroptosis and apoptosis investigated a novel metal-free inducer based on boron and nitrogen co-doped graphdiyne (BN-GDY).¹⁴⁴ This innovative nanozyme exhibited efficient glutathione (GSH) depletion, leading to the induction of ferroptosis by inactivating GSH-dependent peroxidases (GPX4) and promoting apoptosis by downregulating Bcl-2. The high catalytic activity of BN-GDY was supported by kinetic experiments and density functional theory (DFT) calculations. Notably, the research identified a unique Bi-Bi mechanism for the nanozyme's action, as shown in Fig. 12, which differed from the conventional ping-pong Bi-Bi mechanism observed in most peroxidase mimics and natural enzymes. The findings suggested that BN-GDY could serve as a promising therapeutic agent in cancer treatment by leveraging its dual ability to induce both ferroptosis and apoptosis, thereby enhancing cell death in cancer cells.¹⁴⁴ This nonmetal approach to inducing cell death through graphene-based nanozymes offers a novel strategy for nanocatalytic medicine, potentially paving the way for innovative treatments in oncology.

Graphene-based nanozymes represent a compelling frontier in the advancement of cancer therapy. Their multifaceted capabilities, ranging from serving as effective drug delivery vehicles to addressing the challenges posed by the tumor microenvironment, underscore their potential to revolutionize



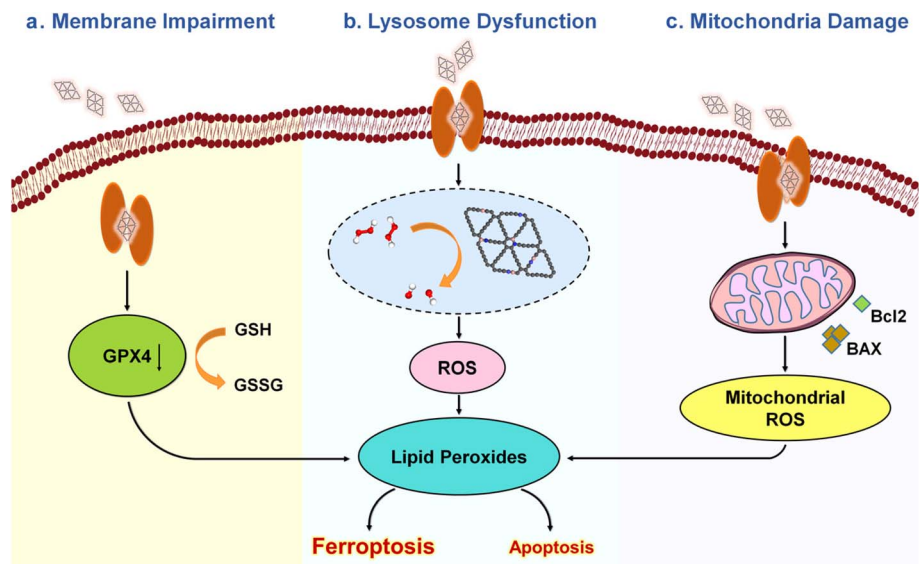


Fig. 12 Illustration of BN-GDY regulation of ferroptosis and apoptosis signal pathways divided into three categories (a) membrane impairment, (b) lysosome dysfunction and (c) mitochondrial damage. Reprinted with permission from ref. 144. Copyright 2022 American Chemical Society.

the landscape of cancer therapeutic strategies. As research in this field continues to evolve, graphene-based nanozymes are poised to play a pivotal role in shaping the future of cancer treatment modalities.

4.6 Miscellaneous biomedical applications

Graphene-based nanozymes are gaining attention for their diverse applications beyond their well-documented biomedical and environmental uses. To illustrate, alcohol intoxication, also known as alcohol poisoning or alcohol overdose, occurs when a person consumes a large amount of alcohol in a short period, leading to a dangerous and potentially life-threatening situation such as steatosis, alcoholic hepatitis, and cirrhosis. Alcohol is a depressant that affects the central nervous system, leading to impaired judgment, coordination, and cognitive function. Alcohol poisoning is a medical emergency that requires immediate attention. It can lead to serious complications, including dehydration, brain damage, and even death. Sun *et al.* developed graphene oxide quantum dots (GOQDs) that effectively mitigated the decline in cell viability caused by ethanol, serving as nanozymes to expedite ethanol metabolism and prevent the buildup of harmful byproducts within cells.²²⁷ GOQDs alleviated the detrimental effects on mitochondria and the excessive production of free radicals. This discovery suggests that GOQDs can mitigate the adverse effects caused by ethanol.²²⁷

A recent study explored the neuroprotective effects of graphene oxide quantum dots (GOQDs) compared to larger graphene oxide (GO) nanosheets, explicitly focusing on their ability to combat oxidative stress and neurotoxicity.²¹⁸ The researchers demonstrated that GOQDs significantly reduced levels of ROS and hydrogen peroxide in PC12 cells exposed to 1-methyl-4-phenyl-pyridinium ion, a commonly used model for neurotoxicity. *In vivo* experiments using zebrafish models revealed that

GOQDs diminished ROS and apoptosis, enhanced locomotive activity, and preserved neuronal structures, indicating superior neuroprotective capabilities. The underlying mechanisms for these effects involved the modulation of metabolic pathways related to antioxidation and neurotransmission, as well as catalase-like activity that aided in the decomposition of H_2O_2 . The study highlighted the potential of biocompatible GOQDs as effective agents for reducing oxidative stress and neurotoxicity, suggesting promising therapeutic applications in neurological health. Overall, the findings emphasized the advantages of using GOQDs over larger GO nanosheets in developing strategies to mitigate neurodegenerative conditions associated with oxidative damage.²¹⁸

5. Challenges and future perspectives

Graphene-based nanozymes, while promising, face several limitations that hinder their practical application. Key challenges include issues with stability and reproducibility, particularly in environments with unstable substrates, such as hydrogen peroxide (H_2O_2), which can lead to inconsistent performance. Additionally, these nanozymes are susceptible to interference from other substances in complex biochemical environments, complicating their use in biosensing applications. Functionalization limitations also pose a challenge, as achieving optimal performance without compromising the unique properties of graphene is crucial. Furthermore, the long-term biocompatibility of these nanozymes remains uncertain, necessitating further research to ensure their safe use in biomedical applications.

To address these challenges, future research should focus on developing more robust formulations and improving functionalization techniques to enhance stability and performance. Comprehensive studies on biocompatibility will be crucial to



ensure the safe application of these technologies in clinical settings. Additionally, prioritizing regulatory compliance and safety assessments will facilitate broader acceptance in various applications. Further exploration of graphene-based nanozymes could lead to the development of even more sensitive and specific diagnostic tools in biosensing. Integrating these nanozymes with advanced technologies, such as microfluidics and wearable devices, could revolutionize point-of-care diagnostics, enabling rapid and accurate testing in various settings. In the realm of catalytic cancer therapy, ongoing studies on graphene-based nanozymes aim to enhance their efficacy and improve their delivery systems. This includes exploring novel targeted delivery methods and synergistic effects combined with other therapeutic approaches. These advancements could eventually lead to more effective and personalized cancer treatments that minimize side effects and improve patient outcomes. The rise of graphene-based nanozymes in biosensing, anti-bacterial, wound healing, and catalytic cancer therapy holds immense potential for advancements in healthcare. Continued research and innovation in this field will likely pave the way for transformative diagnostic techniques and more effective treatments, improving the lives of countless individuals in the future.

6. Conclusion

In conclusion, the emergence of nanozymes, particularly graphene-based nanozymes, has opened up exciting possibilities in the fields of biosensing and catalytic cancer therapy. These innovative nanomaterials exhibit enzyme-like properties, enabling them to mimic the functions of natural enzymes and overcome the limitations of natural enzymes. This review summarizes the synthesis and catalytic properties of graphene-based nanozymes, as well as their applications in biosensing and cancer therapy. Graphene-based nanozymes in biosensing offer great potential for improved diagnostic techniques. Their high sensitivity, selectivity, and stability enable the detection and quantification of various biomarkers with high precision. This has significant implications for early disease detection and the development of personalized medicine. Furthermore, the application of graphene-based nanozymes in catalytic cancer therapy shows promise in revolutionizing cancer treatment. With the ability to target specific cancer cells and trigger catalytic reactions, these nanozymes offer a targeted and efficient approach for destroying cancer cells while minimizing damage to healthy tissues. This could potentially revolutionize cancer treatment by providing more effective and less invasive therapies.

Conflicts of interest

The authors declare that there is no conflict of interest.

Data availability

No primary research results have been included, and no new data has been generated as part of this review.

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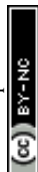
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