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MINI REVIEW

Iron oxide nanoparticles: biosynthesis, peroxidase-like activity, and biosafety

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Abstract

The rising threat of antibiotic-resistant bacterial infections has amplified the demand for alternative therapeutic strategies and efficient catalytic systems. While natural enzymes like horseradish peroxidase offer catalytic potential, their clinical use is limited by instability, high production costs, and environmental sensitivity. Iron oxide nanoparticles (Fe_3O_4 NPs) have emerged as promising alternatives, exhibiting unique physicochemical properties, magnetic responsiveness, biocompatibility, and intrinsic catalytic activity. A key advancement in this field is the adoption of green nanotechnology, which supports the eco-friendly biosynthesis of Fe_3O_4 NPs via biological systems. However, several limitations reduced catalytic activity under certain conditions. This review highlights progress in green synthesis, focusing on iron-resistant and probiotic bacteria as sustainable and scalable biogenic platforms. Compared to chemical methods, these biological routes reduce environmental impact, lower costs, and enhance nanoparticle stability and functionality. This review also addresses the factors influencing the peroxidase-like (POD) activity of NPs. The biomedical relevance of Fe_3O_4 NPs spans diverse applications, including antibacterial therapy, cancer treatment, biosensing, food safety, and enzyme-mimicking catalysis. However, despite their therapeutic promise, significant gaps remain in the biosafety and toxicity assessments of the catalytic activity of Fe_3O_4. Hence, current advancement underscores the underutilized role of bacterial strains in nanoparticle synthesis and identifies critical knowledge gaps that need to be consolidated. It calls for standardized evaluation protocols to support the safe and effective translation of Fe_3O_4 into various applications.

Key points

- *The green synthesis approach of Fe_3O_4NPs offers an eco-friendly route over other methods*
- *Probiotic-mediated synthesis of Fe_3O_4 NPs offers a sustainable and biocompatible approach*
- *Fe_3O_4 NPs mimic POD-like activity for catalytic biomedical and environmental applications*
- *POD-like activity of Fe_3O_4NPs boosts its antibacterial effects via ROS generation*

Keywords Metal oxide nanoparticles · Antibacterial potential · Natural enzyme · Pathogenic bacteria · Peroxidase-like activity · Probiotics

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Introduction

Iron oxide nanoparticles (IONPs) hold great promise in biomedical applications due to their magnetic properties, antibacterial activity, and enzyme-mimetic behavior. Green synthesis methods, which use biological agents, offer a sustainable alternative to conventional approaches by enhancing biocompatibility and reducing toxicity. This review highlights green synthesis, therapeutic and diagnostic potential of IONPs, focusing on eco-friendly synthesis methods, their peroxidase-like (POD) activity, and cytotoxicity, while also addressing related limitations and research gaps.

The overuse of antibiotics has accelerated the emergence of multidrug-resistant (MDR) pathogens, leading to prolonged hospital stays, increased healthcare costs, and elevated mortality (Rana et al. 2023; Shoudho et al. 2024). As conventional antibiotics become less effective, alternative strategies are urgently needed. Metal and metal oxide NPs offer a promising solution due to their unique physicochemical properties and ability to disrupt microbial physiology through membrane damage, oxidative stress, and biomolecular interference (Rana et al. 2023; Correa et al. 2020; Gudkov et al. 2021).

Nanoparticles (NPs), with their small size and large surface area, have emerged as promising candidates for biomedical applications. A wide range of metal and metal oxide NPs has been explored for their potential in antibacterial, antifungal, and anticancer therapies (Waghchaure and Adole 2023). An attractive, eco-friendly, and cost-effective alternative to chemical techniques is the green synthesis approach of Fe_3O_4 NPs by organisms, such as yeast and plant extract (Mahdavi et al. 2013; Namvar et al. 2014; Azizi et al. 2014; Boroumand et al. 2017; Mohd Yusof et al. 2019). Green synthesis reduces the toxic wastes associated with the synthesis of NPs with the chemical approaches, as it does not involve the usage of toxic and harsh chemicals for the synthesis of NPs (Rana et al. 2023). Microorganisms, such as yeast, algae, fungi, and bacteria, may proliferate rapidly in natural conditions. Green synthesis by bacteria reduces the toxic wastes associated with the synthesis of NPs with the chemical approaches, as it does not involve the usage of toxic and harsh chemicals (Hasan et al. 2025; Rabani et al. 2023; Park et al. 2016; Jamzad and Kamari 2020).

Iron oxide nanoparticles (IONPs) exhibit biological benefits, such as antibacterial activity, anti-inflammatory effects, and cancer therapeutic potential (Saxena and Khurana 2020). Their magnetic properties enable targeted drug delivery, while their small size and uniform shape enhance bacterial interactions (Hasan et al. 2025; Stoimenov et al. 2002). IONPs generate reactive oxygen species (ROS), damaging bacterial membranes and causing cell leakage (Fekri Kohan et al. 2023; Waghchaure and Adole 2023; Sangaiya and Jayaprakash 2018; Velayudham and Natarajan 2024). Pathogenic bacteria pose a serious threat to human health due to their ability to initiate infections through a variety of virulence factors, including adhesion molecules, invasion systems, toxins, and capsules, which help them evade host immune responses (Maglangit et al. 2021; Pakbin et al. 2021; Shahrokh et al. 2024; Elias et al. 2024). They are broadly categorized into Gram-positive and Gram-negative types based on their cell wall structures, which also influence their pathogenicity and antibiotic susceptibility (Silhavy et al. 2010).

Gram-positive bacteria (e.g., *Staphylococcus aureus*) with thick peptidoglycan layers remain susceptible to β-lactams but cause persistent infections (Odunitan et al. 2024; Lu et al. 2022). In contrast, Gram-negative bacteria (e.g., *Escherichia coli*) have LPS-rich outer membranes conferring antibiotic resistance (Falagas and Bliziotis 2007; Gontijo et al. 2021). Critically, antibiotic overuse drives resistance via mutations and horizontal gene transfer (Urban-Chmiel et al. 2022; Okaiyeto et al. 2024; Wang et al. 2024). This resistance severely hampers treatment efficacy and increases morbidity and mortality rates (Acar et al. 2024; Munita and Arias 2016).

Fe_3O_4 NPs exhibit antibacterial properties owing to their surface charge and ROS production. The properties of these nanoparticles depend on their synthesis approach, salt precursors, shape, size, and concentration. The size of NPs influences their antibacterial activity and the surface area-to-volume ratio. A study conducted by Golabiazar (2023) revealed that the biologically synthesized Fe_3O_4 NPs by Qazwan Seeds with an average diameter of 14–15 nm exhibited a higher antibacterial potential against gram-negative bacteria rather than gram-positive at the higher concentrations of 20 mg. mL^{-1}.

Another study done by Almutairi et al. (2023) showed that the Fe_3O_4 NPs with an average size of 22.4 nm were synthesized via β-cyclodextrin (β-CD) as a reducing and capping agent and have a remarkable ability as an antibacterial factor against *E. coli* and *B. subtilis* growth. The inhibition zones were 15.0 ± 1.1 and 13.1 ± 2.3 mm, respectively. Research also revealed that the biologically synthesized iron oxide nanoparticles via *Ceratonia siliqua* L. aqueous have antibacterial potentials against Gram-positive (*S. aureus*) and Gram-negative (*E. coli*). The average zones of inhibition were found to be 20.83 ± 0.11 and 24.27 ± 0.12 mm, respectively. These results revealed that these nanoparticles could be utilized as antimicrobial factors, as well as promising candidates in biomedical applications (Aksu Demirezen et al. 2022). The above results indicate that the effectiveness of these nanoparticles is greater against Gram-negative than Gram-positive bacterial strains as a result of the cellular compositions of both groups.

However, the antimicrobial potential and the cytotoxicity of Fe_3O_4 NPs need to be further investigated (Zúñiga-Miranda et al. 2023; Abdoli et al. 2022). Although some of the IONPs show potential for biological applications, clinical acceptance is restricted. Some of these NPs have been withdrawn after approval, highlighting the need to further understand their biomedical applications (e.g., POD activity and antibacterial potential) (Meng et al. 2024). Natural enzymes play a vital role in biological systems and find extensive use in biomedicine; however, their limitations, including inadequate stability and higher costs, have restricted their broader application (Yuan et al. 2025a, b). These problems highlight the need for synthesizing a nanomaterial-based antibacterial agent such as nanozyme (Zhang et al. 2021a, b, c; Liu et al. 2023a, b).

Due to their exceptional magnetic and POD-like activity, nanozymes, particularly Fe_3O_4, have drawn demand (Fu et al. 2023). Since Fe_3O_4 NPs exhibit catalytic characteristics comparable to horseradish peroxidase (HRP), a protein that contains heme and can oxidize different substrates of peroxidase and produce color changes in the presence of H_2O_2, they have been used as peroxidase mimics and have been developed in various domains based on recent research (Ju et al. 2023). The Fe_3O_4 nanozyme has been utilized extensively in cancer treatment (Sharifi et al. 2024; Li et al. 2024), as an antibacterial agent (Wenjun 2021; Xiao et al. 2022), and for biosensing (Guo et al. 2024; Liang et al. 2023), due to their lower cost, high stability, and catalytic activities, and easy to modify its functions compared with the natural enzymes (Liu et al. 2023a, b; Wang et al. 2020a, b).

Different peroxidase substrates can be used (e.g., 3,3′,5,5′-tetramethylbenzidine (TMB), o-phenylenediamine (OPD), di-azo-aminobenzene (DAB), and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)). Fe_3O_4 NPs can catalyze these substrates and convert them to be blue, orange, brown, or green in the presence of H_2O_2. Thus, the biologically synthesized Fe_3O_4 NPs may function as artificial enzymes exhibiting enzymatic characteristics. Nanozyme-based IONPs address several limitations, including inactivation at extreme pH levels, high temperatures, storage challenges, and elevated preparation costs. However, with its easy synthetic process and tunable POD-like activity, Fe_3O_4 nanozyme can serve as a substitute for natural enzymes in various applications. In the presence of H_2O_2, highly toxic ROS are produced by Fe_3O_4 NPs through the production of hydroxyl radicals (•OH) by Fenton-like reactions (Ju et al. 2023; Liu et al. 2019; Mansur et al. 2022).

Interestingly, the POD or catalase-like activity of Fe_3O_4 nanozyme is highly dependent on the pH and other reaction conditions (Mansur et al. 2022). The optimal pH range was observed to be mildly acidic, ~3.5 to physiological ~7.4, and temperature was found to be 37–40 °C (Li et al. 2020; Jiang et al. 2018). Remarkably, Jiang et al. (2018) proposed a crucial method to assess the kinetics and catalytic activity of POD-like nanozymes, including Fe_3O_4, carbon, and gold nanoparticles. They outlined safety protocols, required reagents, and test conditions to guide their biomedical application. Although these nanozymes show similar values of the binding affinities (Km) comparable HRP, their overall catalytic efficiencies differ significantly.

Although the cytotoxicity of bare Fe_3O_4 NPs has been explored, there is a notable gap in research evaluating the biosafety of Fe_3O_4 NPs, especially those exhibiting POD-like activity (Fig. 1). Only limited studies have addressed this issue directly. For instance, L-histidine-functionalized Fe_3O_4 nanozymes and Fe_3O_4@MOF@Au hybrids have shown promising biocompatibility in both in vitro and in vivo models (Yuan et al. 2025a, b; Liu et al. 2023a, b). Despite their encouraging findings, comprehensive toxicological assessments across diverse nanozyme types, doses, and biological systems remain limited. This presents a significant limitation and emphasizes the need for further in-depth studies to ensure the clinical safety and translational potential of Fe_3O_4-based nanozymes.

This mini-review is clearly distinguished from the broader reviews by Ghosh et al. (2022) and Abdoli et al. (2022), which provide general overviews of various nanoparticles synthesized by probiotics (e.g., ZnO, Ag, Au NPs), but lack mechanistic depth and cytotoxicity evaluations. In contrast, our review specifically focuses on Fe_3O_4 NPs synthesized by iron-resistant and probiotic bacteria, highlighting their activity, biomedical potential, and importantly, the critical gap in biosafety and toxicity assessments across concentrations. Unlike Fu et al. (2023), which discusses nanozyme therapies without detailing the impact of the synthesis approach and other factors on activity or clinical limitations, this review offers mechanistic insights into how synthesis parameters influence POD-like activity and evaluate cytotoxicity, making it a novel and targeted contribution to the green nanozyme field.

This review presents a comprehensive analysis of recent progress in the green synthesis, POD-like activity, and biomedical and environmental applications of Fe_3O_4 NPs, with a particular emphasis on their therapeutic potential as well as their biosafety. Special focus is given to their antibacterial mechanisms, particularly in addressing multidrug-resistant pathogens. By critically synthesizing current research, this work underscores the potential of Fe_3O_4 NPs as next-generation enzyme mimics. It highlights key insights into the structure, function relationship, strategies for synthesis optimization, cytotoxicity, and their relevance in clinical applications. Overall, this review bridges the disciplines of nanotechnology and microbiology, supporting the development of Fe_3O_4 NPs-based therapeutics as a promising alternative

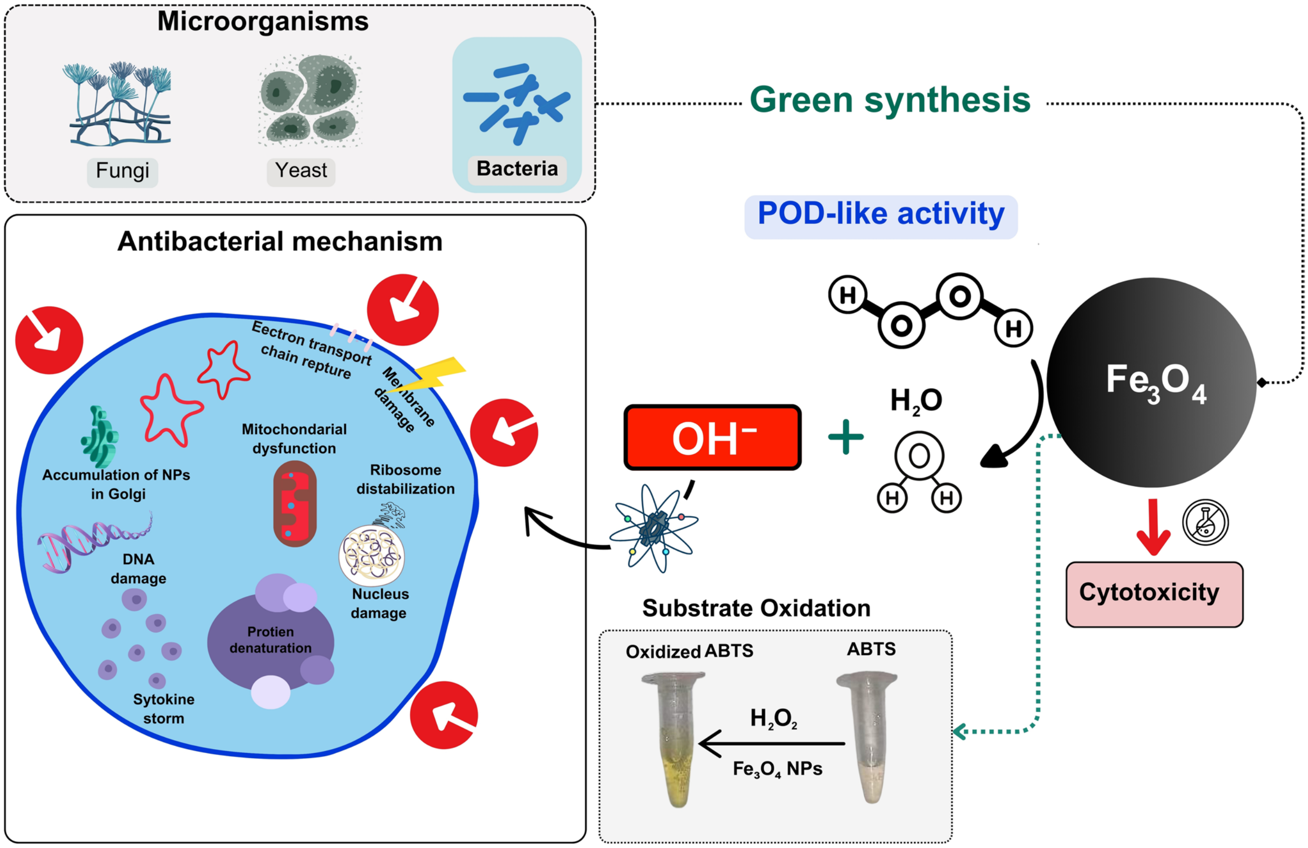

Fig. 1 Graphical abstract for the green synthesis, POD-like activity, and antibacterial potentials of Fe_3O_4 NPs. Created with Canva from https://www.canva.com

to conventional antibiotics and a sustainable substitute for natural enzymes.

Nanomagnetism and size-dependent magnetic behavior of magnetic nanoparticles

Magnetic nanoparticles (MNPs), typically ranging from 1 to 100 nm, are a distinct class of nanomaterials composed of elements like iron, cobalt, and nickel (Sabur and Gafur 2024). Their nanoscale size, comparable to biomolecules, enhances cellular penetration (Kathirvel et al. 2024), while their superparamagnetic behavior enables strong responses to external magnetic fields without residual magnetism (Sabur and Gafur 2024; Kathirvel et al. 2024). These unique properties underpin diverse applications, especially in biomedicine. This property facilitates a wide range of biomedicine applications (Badoni and Prakash 2024; Jiang et al. 2024b), where MNPs are utilized as antibacterial agents and drug delivery (Chota et al. 2023), imaging (Qin et al. 2023), hyperthermia therapies (Al-Samydai et al. 2024), environmental remediation, and data storage technologies (Papolu and Bhogi 2023; Kumar et al. 2023).

Magnetic nanoparticles (MNPs) exhibit unique magnetic behaviors, collectively termed "nanomagnetism," which differ significantly from their bulk counterparts due to quantum mechanical effects such as surface spin disorder, magnetic anisotropy, and size quantization. These properties contribute to enhanced magnetic moments per unit volume and greater coercivity, making MNPs attractive for various applications (Richards et al. 2024; Hao et al. 2010). The superparamagnetism, typically observed in Fe_3O_4 NPs of 10-20 nm, arises when thermal energy exceeds the anisotropy energy barrier, causing rapid fluctuations in magnetic moment orientation. As a result, these particles exhibit zero net magnetization in the absence of an external magnetic field, yet align quickly when one is applied, an attribute beneficial for targeted drug delivery and magnetic imaging (Wu et al. 2019a, b). Although saturation magnetization (Ms) in Fe_3O_4 NPs can reach high values under an applied field, it drops to zero upon field removal due to thermal relaxation (Lu et al. 2010; Rani and Varma 2015).

The small size of MNPs enhances the surface-to-volume ratio, facilitating chemical functionalization while simultaneously influencing magnetic performance. A decrease in particle size leads to reduced magnetism, primarily due to increased spin disorder at the surface, dipolar interactions, structural defects, and incomplete crystallization, all of which disrupt magnetic domain alignment (Scurti et al. 2022; Daoush 2017; Roy et al. 2004; Mehdipour et al. 2021; Wu et al. 2019a, b). Surface modification of MNPs with ligands, polymers, or functional groups extends their application in drug delivery, biosensing, and catalysis (Oliveira et al. 2024; Wu et al. 2016; Zhang et al. 2019).

Fe_3O_4 NPs are among the most widely studied and utilized magnetic nanomaterials, owing to their cost-effectiveness, biocompatibility, and superior magnetic properties (Kumar et al. 2024). Their physical and chemical behavior is strongly influenced by morphological features such as size (Ba-Abbad et al. 2022). Naturally occurring Fe_3O_4 NPs, first identified in the Magnesia region of Asia Minor, contain both ferrous (Fe^{2+}) and ferric (Fe^{3+}) ions, resulting in a mixed-valence structure that imparts distinctive magnetic characteristics. This unique configuration underpins its wide applicability in fields such as biomedicine, catalysis, and environmental remediation (Rukhsar et al. 2022; Boxall et al. 1996).

Diverse synthesis techniques, electrochemical (Wang et al. 2007; Hachem et al. 2022), microwave (Wang et al. 2021), and green synthesis approaches (Fahim et al. 2024), enable the production of MNPs using various magnetic materials, including Fe, Co, Ni, Au, Ti, and their oxides or ferrites. Among these, magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) are the most extensively utilized in therapeutic contexts (Chavan et al. 2022). Transitioning from bulk to nanoscale form markedly alters the structural, electrical, and magnetic properties of Fe_3O_4 NPs, enhancing their performance in advanced applications. The synthesis route plays a critical role in determining the physicochemical characteristics of Fe_3O_4 NPs, which in turn affect their stability, dispersion, and functional performance. A simplified reaction for magnetite formation is represented in the following equation:

$$FeO + Fe_2O_3 \rightarrow Fe_3O_4$$

This equation represents the oversimplification of the reaction synthesis of magnetite (Rukhsar et al. 2022; Boxall et al. 1996). Table 1 provides a comparative summary of various Fe_3O_4 NPs synthesized through different methods, highlighting their magnetic properties, particle sizes, saturation magnetization (Ms), and Functional applications. The nanoparticles exhibit superparamagnetic behavior with sizes ranging from 8 to 300 nm and Ms values between approximately 19 and 72.4 emu. g^{-1}. Their reported applications include magnetic hyperthermia, heavy metal ion adsorption, antibacterial activity, and anticancer therapy.

Emerging and conventional methods for the synthesis of Fe_3O_4 nanoparticles

The synthesis approaches play a pivotal role in determining the physicochemical properties, morphology, stability, and functional performance of nanoparticles, thereby influencing their suitability for specific applications (Al-Harbi and Abd-Elrahman 2024; Dixit et al. 2022; Osman et al. 2024; Vijayaram et al. 2024; Kumar et al. 2021). This section provides a comparative overview of conventional chemical and green synthesis methods, discussing their respective advantages and limitations. Nanoparticle synthesis strategies are broadly categorized into two fundamental approaches, including top-down and bottom-up (Kathirvel et al. 2024; Banjara et al. 2024) (Fig. 2). Top-down approaches involve the mechanical or physical breakdown of bulk materials into nanoscale particles. These include methods like grinding, milling, laser ablation, lithography, pyrolysis, sputtering, and physical vapor deposition (PVD). In this process, the bulk materials first undergo energy input, such as mechanical force or thermal energy, leading to their conversion into fine powders. The particles are then subjected to mechanical breakdown to attain the nanoscale.

This method is comparatively rapid and scalable. Nonetheless, it often entails significant energy consumption, the creation of surface flaws, and a wider size dispersion of the resultant nanoparticles (Tripathy et al. 2023; Messaoudi and Bendahou 2020) (Fig. 3). Conversely, bottom-up approaches synthesize nanoparticles from atomic or molecular precursors by processes such as chemical vapor deposition (CVD), reduction, sol-gel processes, and biological procedures involving plant extracts and microbial synthesis. Nucleation and growth at the atomic or molecular level result in cluster formation, which then condenses into nanoparticles. In several instances, ionization via energy input is essential for beginning reactions or promoting condensation, allowing atoms or molecules to surmount energy barriers and create stable clusters that develop into well-defined nanoparticles (Fig. 3). This "building-up" strategy allows better control over particle size and homogeneity. While top-down techniques are suitable for producing large quantities, they may result in less uniformity. Bottom-up methods, especially green synthesis routes, offer eco-friendly alternatives with enhanced control over nanoparticle characteristics but often require more complex optimization (Khan et al. 2024; Xu et al. 2024).

Chemical synthesis

Chemical synthesis remains one of the most widely used and efficient methods for producing nanoparticles due to its simplicity, scalability, cost-effectiveness, and rapid production rates without the need for sophisticated

Table 1 Comparative summary of superparamagnetic nanoparticles with their synthesis routes, magnetic properties, and applications

Type of MNPs		Synthesis method	Size (nm)	Saturation magnetization (emu. g^{-1})	Applications	Ref
IONPs		Green synthesis via *Sargassum* spp	23.60 ± 0.62	65.85	Wastewater treatment	Bhole et al. (2023)
Fe_3O_4 NPs		Green synthesis via *Citrus sinensis* peel	23.4	-	Hyperthermia	Eldeeb et al. (2023)
Fe_3O_4 NPs		Chemical	15	~40	Hyperthermia	Lemine et al. (2023)
NFO/Au NPs		Chemical	NFO (50–100 surrounded with AuNPs (10–30 nm)	Upon coating, it was slightly decreased from 33 to 30 emu. g^{-1}	Removal of oxytetracycline antibiotics	Nguyen et al. (2024)
Fe_3O_4 NPs		Solvothemal	200–300	72.4	-	Ni et al. (2022)
IONPs		Chemical	11.61	72.75	Cancer treatment	Peréz et al. (2024)
Fe_3O_4		Co-precipitation	8–55	-	Apoptosis in the human colorectal adenocarcinoma cell line	Ranjbary et al. (2023)
Fe_3O_4		Solvothemal	20.6	65.1	Antibacterial	Rong et al. (2024)
$La_{0.8}Sr_{0.2}MnO_{3-\delta}$		Conventional solid-state reaction method	8 to 53	~55 to ~19 (Decrease with increase in size)	-	Roy et al. (2004)
Fe/Au NPs (1:1, 2:1, and 3:2 ration)		Chemical method	31, 38, and 36	165	Hyperthermia	Sanad et al. (2021)
Fe_3O_4, MEG, VbMEG, and pVbMEG		Co-precipitation	12.4, 7.3, 9.2, and 9.7	53.4, 21.4, and 38.3	Adsorption of metal anions	Scurti et al. (2022)
$NiFe_2O_4$ NPs		An in-liquid plasma method	~10	42.27	-	Shabani et al. (2021)
Fe_3O_4 NPs		Green synthesis via *Shewanella putrefaciens*	6 ± 2	73.8	-	Parthasarathy and Chandra (2024)
Coated $NiFe_2O_4$ NPs	-Citric acid -Urea -Sodium azide -Glycin	Sol–gel	41 25 25 24	37.80 47.44 42.73 48.24	-	Bajaj et al. (2020)
$Mn_{0.9}Mg_{0.1}Fe_2O_4$ NPs		Green synthesis	13.66 ± 2.05	15.28	Antibacterial and antioxidant	Salih (2024)
Fe_3O_4 and Fe_2O_3		Ziziphus lotus leaf extract	34 and 54	-	Antimicrobial activity and MB degradation	Abbas et al. (2025)
Fe_2O_3 NPs		*Arthrospira platensis* extract	-	-	Antimicrobial activity	Alprol et al. (2025)

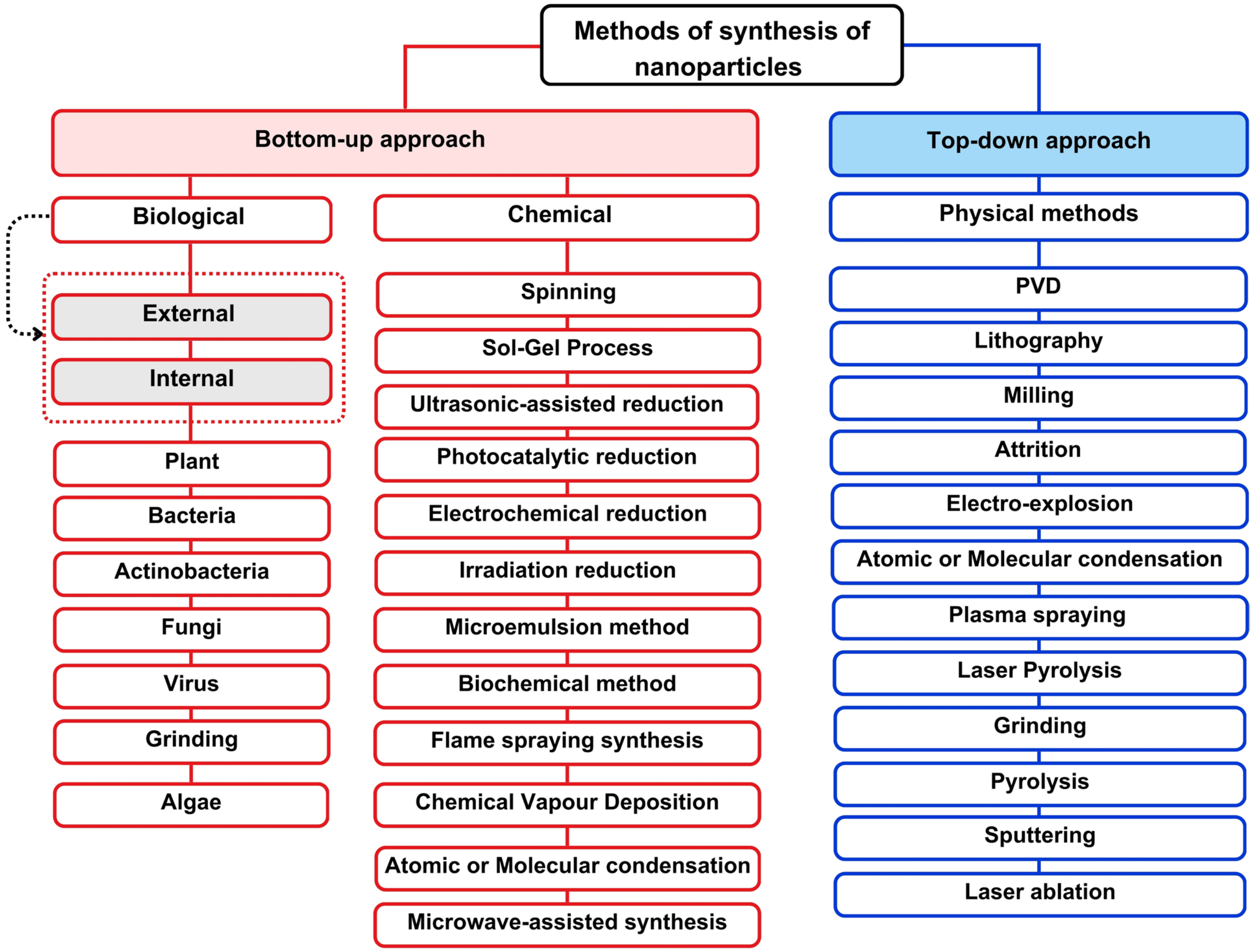

Fig. 2 Various methods for the synthesis of nanoparticles. Created with Canva from https://www.canva.com

equipment. These methods allow for the generation of stable nanoparticles with extended shelf-life and minimal degradation (Szczyglewska et al. 2023). However, despite these advantages, chemical approaches present notable drawbacks. The frequent use of toxic reducing agents, stabilizers, and organic solvents raises significant environmental and health concerns.

Additionally, the risk of nanoparticle contamination with residual chemicals and the generation of harmful by-products limit their biocompatibility and sustainability (Anwar 2018; Altammar 2023; Szczyglewska et al. 2023; Shekhar et al. 2023; Rani et al. 2023). These limitations have prompted increasing interest in alternative eco-friendly synthesis techniques, such as green synthesis methods, which aim to enhance safety while maintaining nanoparticle efficacy and stability.

Biological synthesis by iron-resistant bacteria

Iron is a vital micronutrient for bacterial survival and functions such as colonization, growth, and pathogenicity. To acquire iron from their surroundings, bacteria have evolved intricate mechanisms, including the secretion of enzymes and metabolites that reduce iron salts to bioavailable forms (Huo et al. 2021). Notably, several microorganisms exhibit inherent metal-reducing and detoxifying capabilities. By utilizing enzymes, cofactors, and secondary metabolites, they can transform ionic iron precursors into IONPs (Singh et al. 2016; Priya et al. 2021; Zúñiga-Miranda et al. 2023).

Species such as *Klebsiella pneumoniae*, *Bacillus cereus*, *B. tropicus*, *Sporosarcina luteola*, *K. africana*, *B.*

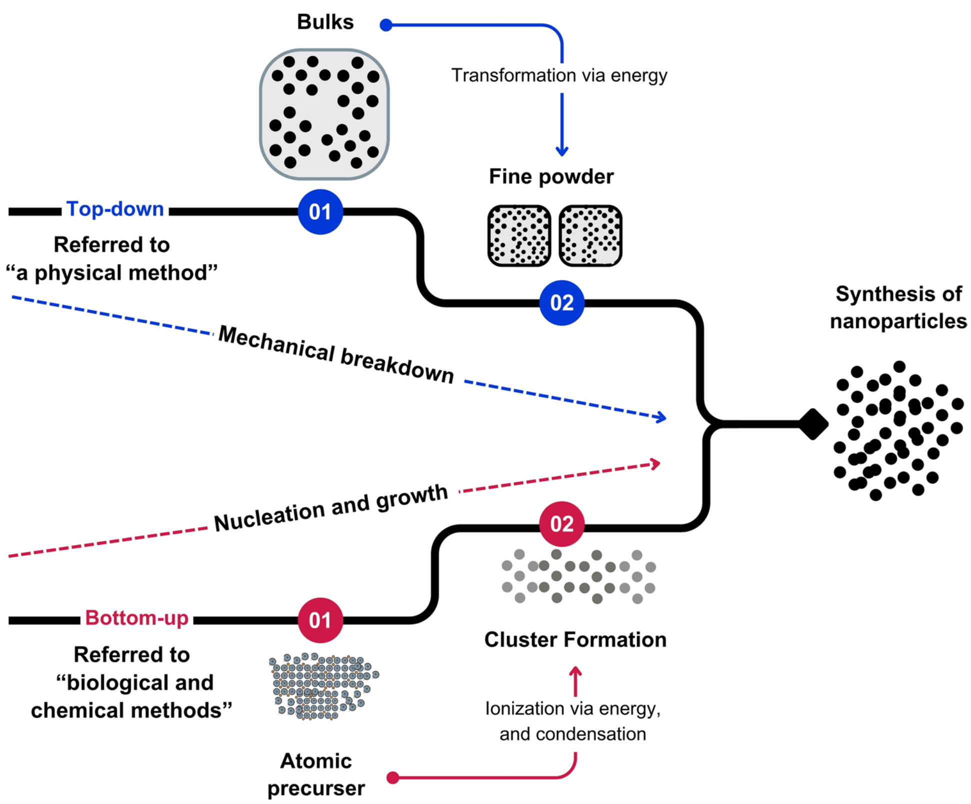

Fig. 3 Diagrammatic illustration of top-down and bottom-up approaches for the synthesis of nanoparticles. Created with Canva from https://www.canva.com

subtilis, *K. quasipneumoniae*, and *B. badius* have been reported to possess ferric-reducing capabilities for the biosynthesis of iron oxide nanoparticles (Daramola et al. 2024). Additionally, proteins containing functional groups such as hydroxyl, carboxyl, thiol, and amine contribute to nanoparticle stabilization by binding metal ions and enabling their reduction (Priya et al. 2021; Anil Kumar et al. 2007; Ishak et al. 2019). Other contributing biomolecules include ferric reductase (Alam et al. 2020; Pal et al. 2023), hydrogenases, and antioxidant enzymes (Pal et al. 2023). These interactions occur at the microbial surface (cell wall or periplasm), where the metal ions are reduced and nucleate into nanoparticles (Lahiri et al. 2021). Interestingly, a study conducted by Hasan et al. 2025 reported for the first time the ability of an iron-tolerant bacterium (*Bacillus proteolyticus* UPMC1508) to biologically synthesize Fe_3O_4 NPs with controlled size and biocompatibility.

The morphology and size of nanoparticles significantly influence their physicochemical behavior, including surface area, optical properties, and reactivity. Controlling these parameters is crucial for tailoring nanoparticles for specific applications (Tahir et al. 2021; Ashrafi-Saiedlou et al. 2025). Green synthesis using microorganisms offers an eco-friendly, cost-effective, and scalable route for producing nanoparticles with controlled morphology and size (Singh et al. 2016; Sindhwani et al. 2020).

The green synthesis of NPs occurs via two main mechanisms, including extracellular and intracellular mechanisms (Zakariya et al. 2022). Extracellular involves the reduction of metal ions by secreted enzymes or proteins in the supernatant. These biomolecules act as both reducing and stabilizing agents, with nanoparticle formation typically indicated by a color change in the medium (Fatemi et al. 2018; Rabani et al. 2023). Metal ions may also bind electrostatically to the microbial surface before enzymatic reduction (Lahiri et al. 2021; Zúñiga-Miranda et al. 2023).

Intracellular synthesis entails the uptake of metal ions through electrostatic interactions with carboxyl groups on the cell wall. The ions are then reduced by intracellular enzymes, leading to nanoparticle formation within the cytoplasm. This process involves culturing microorganisms, harvesting and washing the biomass, and incubating it with metal salt solutions (Nadeem et al. 2021, 2022). Table 2 highlights that extracellular bacterial synthesis is among the most efficient and scalable green approaches for synthesizing Fe_3O_4 NPs owing to its simplicity, sustainability, and ease of nanoparticle recovery. It also illustrates the particle size, morphology, precursor salts, and various analytical techniques that were employed across the reviewed studies for measuring the diameter of biosynthesized Fe_3O_4 NPs. These include transmission electron microscopy (TEM) for morphological analysis and particle size estimation, scanning

Table 2 The biological synthesis of Fe_3O_4 NPs along with their morphologies and type of precursor salt used

Bacterial strain	Mechanism of synthesis	Size (nm) and instrument used	Morphology	Salt type	Applications	Ref
-*B. pasteurii* -*B. licheniformis* -*B. subtilis*	Extracellular	−37–97 -TEM	Rhombohedral	FeCl $FeCl_3$	Environmental remediation	Daneshvar and Hosseini (2018)
B. circulans	Extracellular	−18.37 -SEM	Spherical	$FeSO_4.7H_2O$	Antioxidant	Rabani et al. (2023)
B. coagulas	Extracellular	−15.2 -XRD	Irregular cubic	$FeCl_2.4H_2O$	Antibacterial and antibiofilm activity	Al-Maliki and Taj-Aldeen (2021)
B. megaterium	Extracellular	- 20–30 -FE-SEM	Spherical	$FeSO_4{\cdot}7H_2O$	Antibacterial	Hajiali et al. (2025)
B. subtilis	Extracellular	−30–40 -XRD	Spherical	-	Enhancing crop growth	Naik et al. (2025)
B. subtilis	Extracellular	−55–80 -SEM	Cubic and spherical	Fe_2O_3	Antibacterial	Sasani et al. (2021)
Bacillus proteolyticus	Extracellular	~5	Spherical	$FeCl_3{\cdot}6H_2O$	-	Hasan et al. (2025)
Endophytic *B. siamensis* (MT052693)	Extracellular	−34 -FE-EM	Cauliflower	Fe_2O_3	-	Ashengroph and Meschi (2022)
Gluconacetobacter xylinus	Intracellular	−50 -SEM	-	-$FeCl_3{\cdot}6H_2O$ -$FeCl_2{\cdot}4H_2O$	Bioengineering	Arias et al. (2016)
Lactobacillus fermentum	Intracellular	−10–15 -TEM	Spherical	iron sulfate solution (III)	-	Fani et al. (2018)
Proteus mirabilis	Intracellular	−1.44–1.92 -TEM	Spherical	Fe $(NO_3)_3.9H_2O$	Disinfection	Zaki et al. (2019)
Pseudomonas	Extracellular	−23 -TEM	Spherical	-$FeSO_4{\cdot}6H_2O$ -$FeCl_3{\cdot}6H_2O$	Anticoagulant	Crespo et al. (2017)
Shewanella putrefaciens CN-32	Extracellular	-6 ± 2 -XRD	Pseudo-spherical	$FeCl_3{\cdot}6H_2O$	-	Parthasarathy and Chandra (2024)
Thermoanaerobacter sp	Extracellular	-13.1 ± 0.8 -TEM	Spherical	-	-	Moon et al. (2010)
Thermophilic *B. tequilensis* ASFS.1	Extracellular	−10–40 -FE-SEM	Spherical	-	Anticancer	Satarzadeh et al. (2024)
Thiobacillus thioparu	Intracellular	-	-	$FeSO_4$	-	Elcey et al. (2014)

electron microscopy (SEM) and field emission scanning electron microscopy (FE-SEM) for surface topography, and X-ray diffraction (XRD).

The possible mechanisms for the biosynthesis of Fe_3O_4 nanoparticles

Bacteria play a pivotal role in the green synthesis of Fe_3O_4 NPs through diverse mechanisms, including extracellular precipitation, intracellular reduction, and efflux-mediated pathways. These adaptive strategies enable survival in iron-rich environments while facilitating the transformation of various iron precursors, such as $FeCl_3{\cdot}6H_2O$, $FeCl_3$, $Fe(NO_3)_3.9H_2O$, $FeCl_2.4H_2O$, $FeSO_4.5H_2O$, and other sulfate salts, into IONPs (Priya et al. 2021).

Extracellular biosynthesis is often mediated by bacterial secretion of redox-active compounds (e.g., flavins and quinones), which serve as electron shuttles for Fe^{3+} reduction. Strains such as *Magnetospirillum gryphiswaldense* and *Shewanella putrefaciens* CN-32 have demonstrated the ability to synthesize Fe_3O_4 NPs through extracellular electron transfer pathways (Rosenfeldt et al. 2021; Parthasarathy and Chandra 2024). Additionally, dissimilatory iron-reducing bacteria, including *Shewanella*, *Geobacter*, and *Thermoanaerobacter* spp., precipitate magnetite as a metabolic byproduct by coupling Fe^{3+} reduction with the oxidation of organic substrates (Daramola et al. 2024). This extracellular approach is advantageous due to simplified downstream processing, scalability, and lower toxicity risks, making it suitable for industrial applications (Parthasarathy and Chandra 2024).

In contrast, intracellular biosynthesis involves enzymatic reduction pathways within bacterial cells. Enzymes such as ferric reductases, Fe(III)-EDTA reductases, and hydrogenases catalyze the reduction of Fe^{3+} to Fe^{2+}, leading to nanoparticle nucleation in the periplasm or cytoplasm. Microorganisms such as *Paracoccus denitrificans* and various *Bacillus* species are known to mediate this intracellular route effectively (Parthasarathy and Chandra 2024; Daramola et al. 2024). The efficiency of nanoparticle synthesis is influenced by several variables, including strain type, cell wall functional groups (e.g., -CHO, -CO, -COOH, -OH, -C-O-C, -C≡C), precursor concentration, temperature, pH, and nutrient medium composition. These factors govern critical nanoparticle attributes such as size, morphology, and stability (Iravani and Varma 2020; Priya et al. 2021; Mohammadpour et al. 2023; Liu et al. 2023a, b).

Despite the promise of microbial synthesis for environmentally sustainable nanomaterials, Fe_3O_4 NPs commercial translation remains Limited. Only about 1% of bacteria-mediated nanoparticle systems have reached industrial deployment, constrained by the need for strict growth conditions, sensitivity to environmental parameters, and complex scale-up requirements (Kapoor et al. 2021; Atalah et al. 2022). In conclusion, extracellular synthesis of Fe_3O_4 NPs offers significant advantages over intracellular methods, particularly in the context of large-scale and industrial applications. A key benefit is the simplified recovery process, as nanoparticles are directly secreted into the extracellular medium, eliminating the need for labor-intensive cell disruption and purification steps associated with intracellular synthesis. This not only enhances process efficiency but also supports scalability and cost-effectiveness, making extracellular biosynthesis a more practical and sustainable approach for industrial nanoparticle production.

Mechanistic roles of probiotic bacteria in the biosynthesis of nanoparticles

The term probiotic, meaning "for life," was first introduced by Werner Kollath in 1953 and later refined to refer to live microorganisms that confer health benefits to the host when administered in adequate amounts, as defined by the World Health Organization (WHO). Probiotic formulations typically contain beneficial bacteria that help maintain the balance of intestinal Microbiota. The recommended effective dose ranges from 10^7 to 10^9 colony-forming units (CFU) per oral administration (Gasbarrini et al. 2016; Abdoli et al. 2022; McFarland 2015; Sanders 2008).

Probiotics have been extensively studied for their therapeutic potential in a variety of health conditions. They are known to support gastrointestinal health by alleviating disorders such as inflammatory bowel disease (IBD) (Li et al. 2023), irritable bowel syndrome (IBS) (Simon et al. 2021), and peptic ulcers (Bai et al. 2022). Additionally, probiotics contribute to the management of chronic intestinal inflammation and have shown effectiveness in reducing diarrhea, urinary and genital tract infections, and atopic dermatitis (Manzoor et al. 2022). Their benefits also extend to cardiovascular health and the prevention of colorectal cancer, with studies suggesting a reduced risk of colon cancer following probiotic (Deng et al. 2023; Budu et al. 2022). A wide range of bacterial and yeast strains are utilized as probiotics, each exhibiting strain-specific health-promoting properties (Abdoli et al. 2022).

Probiotic bacteria such as *Lactobacillus*, *Bifidobacterium*, and *Lactococcus*, classified as Generally Recognized As Safe (GRAS), are not only resilient to harsh gastrointestinal environments (e.g., low pH and bile salts) but are also gaining attention for their role in green nanomaterial synthesis (Liang et al. 2024; Khushboo and Malik 2023). Several strains, including *Lactobacillus plantarum*, *L. acidophilus*, *L. casei*, *L. fermentum*, and *Bacillus licheniformis*, have successfully biosynthesized diverse nanoparticles such as gold (AuNPs) (Dhandapani et al. 2024), silver (AgNPs), selenium (SeNPs) (Alam et al. 2020), zinc oxide (ZnONPs) (Mohd Yusof et al. 2019), and Fe_3O_4 NPs (Fani et al. 2018; Torabian et al. 2018), demonstrating their versatility and eco-compatibility. Despite these advances, the nanobiotechnological applications of probiotic microflora remain underexplored (Ghosh et al. 2022).

A key limitation in probiotic-mediated synthesis is the frequent production of anisotropic and polydispersed nanoparticles, which hampers reproducibility and performance in biomedical applications (Ghosh et al. 2022). Given that nanoparticle size and morphology critically affect their functionality, fine-tuning parameters, such as pH, metal salt concentration, culture age, inoculum size, and temperature, are essential to achieve uniform, monodispersed nanoparticles (Ranpariya et al. 2021; Bloch et al. 2021). Probiotic bacteria can mediate nanoparticle biosynthesis through both intracellular and extracellular mechanisms. Functional biomolecules such as enzymes, peptides, sugars, and proteins act as reducing and stabilizing agents, eliminating the need for toxic chemicals. Mechanistic pathways include extracellular secretion of metabolites like lacto-N-triose, membrane lipid-mediated reduction via diglycosyldiacylglycerol (DGDG) and triglycosyldiacylglycerol (TGDG), NADH-dependent enzymatic reduction at the membrane, and capsular polysaccharide-mediated capping and reduction, involving sugars like galactose and rhamnose. These biosynthetic routes highlight the multifunctional role of probiotics in nanoparticle formation and stabilization, underscoring their promise as biocompatible, cost-effective, and sustainable nanofactories for future biomedical and industrial applications (Ghosh et al. 2022).

Nanozymes as promising alternatives to natural enzymes

Natural enzymes are highly specialized biological macromolecules, primarily proteins, that catalyze biochemical reactions with exceptional specificity and efficiency under mild physiological conditions (Shahraki 2022; Shahraki et al. 2024). Among them, oxidoreductases form a major enzyme class responsible for catalyzing oxidation-reduction (redox) reactions vital to metabolic, environmental, and industrial processes (Cárdenas-Moreno et al. 2023; Younus 2019). Key subclasses of oxidoreductases include oxygenases, oxidases, dehydrogenases, and, most notably, peroxidases (PODs). Peroxidases catalyze the reduction of hydrogen peroxide (H_2O_2) and organic peroxides using various electron donors such as phenols and amines (Zhang et al. 2021a, b, c). They are ubiquitously distributed across plants, animals, and microorganisms, where they play essential roles in oxidative stress response, hormone metabolism, defense systems, and redox regulation (Veitch 2004).

Due to their broad substrate specificity and catalytic adaptability, peroxidases have widespread applications in environmental science; they contribute significantly to pollutant degradation and bioremediation (Bilal et al. 2017). In biomedical contexts, peroxidases are valued for their roles in therapeutic strategies, particularly in antibacterial (Zhang et al. 2021a, b, c) and anticancer treatments (Brück and Brück 2011). They also serve as effective biocatalysts in industrial biotechnology, where they are employed in green synthesis, bioconversion processes, and enzyme-based production systems (Krainer and Glieder 2015). Additionally, peroxidases are widely utilized in medical diagnostics, notably in glucose detection assays and enzyme-linked immunosorbent assays (ELISA), owing to their high specificity and catalytic efficiency (Liu et al. 2022a, b) (Fig. 4).

While natural enzymes are vital biocatalysts in living systems, their application beyond physiological contexts, such as in industrial, medical, or environmental settings, is constrained by several limitations. These include high production and purification costs, low stability under harsh conditions (e.g., pH and temperature), complex preparation, poor recyclability, limited targeting ability, and potential immunogenicity (Shahraki et al. 2024; Zhang et al. 2022; Moradi Hasan-Abad et al. 2024). These drawbacks have driven interest in nanozymes, synthetic nanomaterials that mimic enzyme-like activities and offer enhanced durability, reusability, and cost-effectiveness.

The term "nanozyme," a blend of "nano" and "enzyme," analogous to "ribozyme" and "DNAzyme," refers to nanostructured materials with intrinsic catalytic properties (Shahraki et al. 2024). The concept originated with Manea et al. (2004), who engineered gold nanoparticles to mimic transphosphorylation reactions. A significant breakthrough came from Gao et al. (2007), who demonstrated that Fe_3O_4 NPs exhibit POD-like activity, catalyzing the transformation of substrates in the presence of H_2O_2, thus establishing a new class of artificial enzyme mimics.

Since the original discovery of nanozyme activity, researchers such as Wei and Wang (2013) have expanded the concept to encompass a broad spectrum of nanomaterials exhibiting catalytic behavior. Today, the terms "enzyme-like" and "enzyme-mimicking" are used to differentiate nanozymes based on whether they simulate enzyme function mechanistically or structurally (Zandieh & Liu, 2024). This evolving framework supports both fundamental mechanistic insights and diverse practical applications, creating a feedback loop of innovation in nanozyme research.

Nanozymes offer distinct advantages over natural enzymes and traditional synthetic catalysts. They are more cost-effective, easier to synthesize, highly stable under extreme conditions (e.g., pH and temperature), and amenable to large-scale production. These attributes make them highly attractive for applications across biomedicine, environmental remediation, and industrial (Wei et al. 2021; Fan et al. 2023; Li et al. 2022). Their multifunctionality and resilience have made nanozymes a rapidly growing topic in catalytic and materials science.

Most nanozymes mimic the catalytic behavior of oxidoreductase enzymes, which mediate oxidation–reduction (redox) reactions vital to physiological functions. Commonly mimicked oxidoreductases include oxidase (OXD), catalase (CAT), peroxidase (POD), and superoxide dismutase (SOD) (Shahraki et al. 2024). Remarkably, a single nanozyme can exhibit multi-enzyme activity, and its catalytic efficiency can be modulated through surface modification or structural engineering, enhancing substrate specificity and reaction rate. Nanozymes have shown tremendous potential in both diagnostic and therapeutic fields. In diagnostics, they are widely employed in biomarker detection and imaging (Wang et al. 2023a, b; Qin et al. 2023), as well as in biosensing systems such as fluorescent-based (Wang et al. 2023a, b; Mu et al. 2023), electrochemical (Yang et al. 2024a, b), colorimetric (Yang et al. 2024a, b), and lateral flow assays (Wu and Kamruzzaman 2024). In the therapeutic context, nanozymes have been investigated for their antioxidant and anticancer properties (Zhong et al. 2023), their effectiveness in cardiovascular diseases and sepsis treatment, and their role in stem cell regulation (Jiang et al. 2024a; Zhang et al. 2023; Hou et al. 2024). Additionally, they have demonstrated promising antibacterial and wound healing activities (Shan et al. 2023). In conclusion, these applications highlight the multifunctionality of nanozymes and underscore their emerging significance in advanced biomedical technologies.

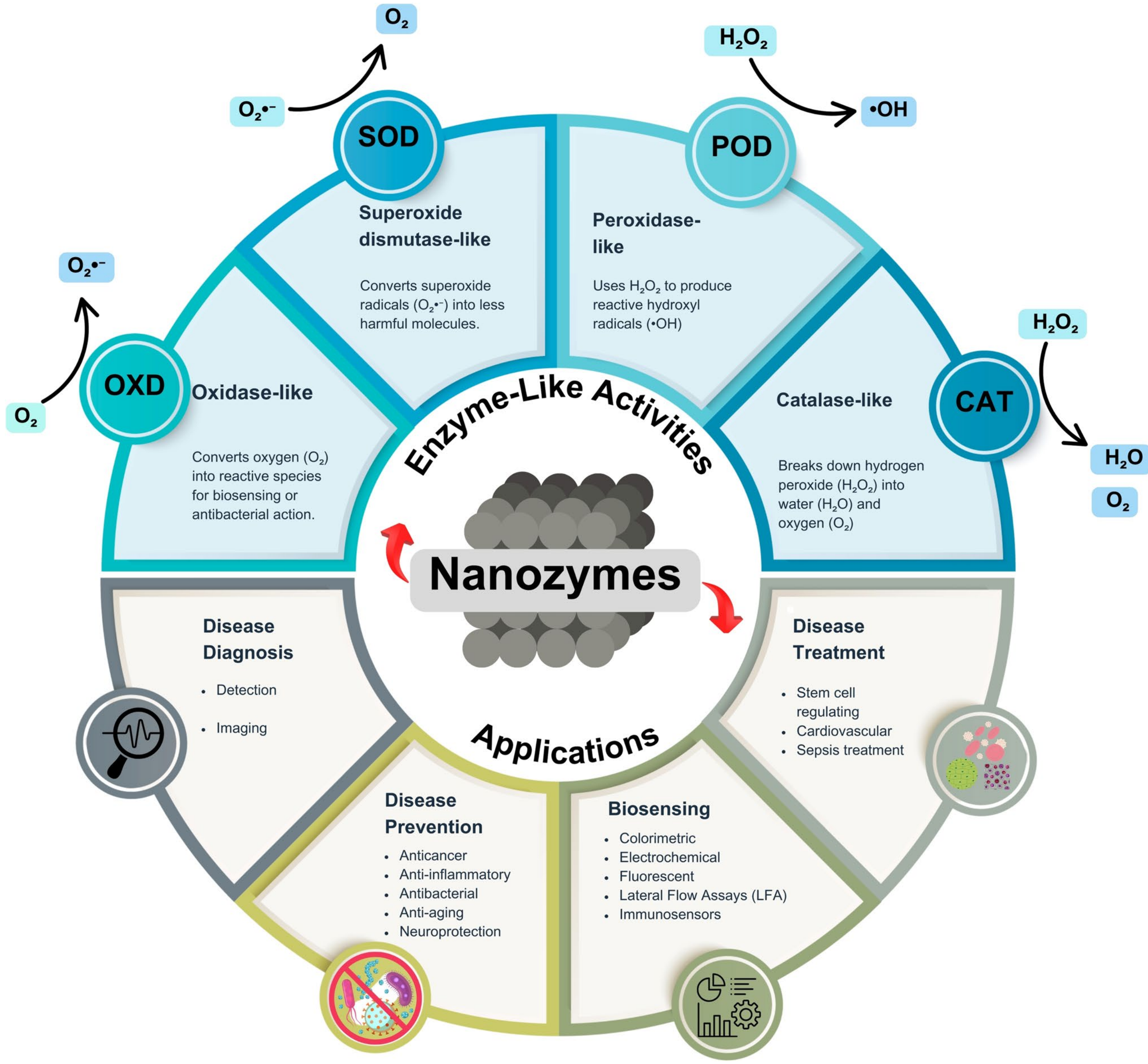

Fig. 4 The enzymatic-mimicking activities of nanozyme and their applications. Created with Canva from https://www.canva.com

Iron oxide nanoparticles as peroxidase mimics

In a landmark study, Gao et al. (2007) discovered that Fe_3O_4 NPs exhibit intrinsic POD-like activity comparable to HRP. In the presence of H_2O_2, Fe_3O_4 NPs catalyze the oxidation of chromogenic substrates such as TMB, ABTS, and OPD, producing a visible color change. This catalytic reaction follows Michaelis-Menten kinetics and is influenced by pH and temperature, confirming the behavior of an enzyme-mimetic of nanozyme under optimal conditions. Beyond catalysis, Fe_3O_4 NPs possess optical, magnetic, and electrical properties, making them highly versatile in diagnostic and biomedical applications (Guo et al. 2024; Dong et al. 2022; Tang et al. 2021).

Unlike natural enzymes, Fe_3O_4 nanozymes offer enhanced stability, lower synthesis costs, and resistance to denaturation under extreme pH or temperature (Liu et al. 2019; Guo et al. 2024). While the complete mechanistic details remain under investigation, their catalytic activity is generally attributed to redox cycling between Fe^{2+} and Fe^{3+} ions, which decomposes H_2O_2 and generates highly reactive hydroxyl radicals (·OH) via Fenton or Haber-Weiss reactions (Guan et al. 2024; Shi et al. 2014).

Mechanistic studies have shown that the electron transfer through Fe^{2+}-O-Fe^{3+} chains within the crystal lattice of nanoparticles plays a crucial role in sustaining catalytic turnover. Surface Fe^{2+} regeneration enables continuous H_2O_2 decomposition, while prolonged oxidation leads to Fe^{2+} depletion, resulting in a phase transition from Fe_3O_4 (magnetite) to γ-Fe_2O_3 (maghemite), a less active form due to reduced Fe^{2+} content. In this process, TMB undergoes oxidation initiated by ·OH radicals, transforming from its diamine state into a charge-transfer complex known as oxidized TMB, which produces a characteristic blue-green color. This complex consists of both the partially oxidized and fully oxidized diimine (TMB^{2+}) forms. A similar colorimetric oxidation mechanism occurs with ABTS, reinforcing the diagnostic utility of Fe_3O_4 nanozymes (Han et al. 2022; Dong et al. 2022). Figure 5 illustrates this catalytic mechanism and the associated phase transition of Fe_3O_4 nanozymes.

The limitations and factors influencing the peroxidase-mimicking efficiency of Fe_3O_4 nanoparticles

Fe_3O_4 nanozymes hold significant potential for biomedical applications, particularly in therapeutic platforms, due to their POD-like catalytic activity and ability to generate ROS (Feng et al. 2022a, b; Tang et al. 2021). However, several studies have reported inconsistent or reduced catalytic performance under certain conditions. A key limiting factor is structural phase transformation, wherein prolonged catalytic activity leads to the oxidation of Fe_3O_4 to γ-Fe_2O_3, a less active phase with diminished POD-like activity due to the depletion of Fe^{2+} ions, essential for Fenton-like reactions that generate hydroxyl radicals (·OH), the main oxidizing agents in substrate conversion (Dong et al. 2022; Tang et al. 2021).

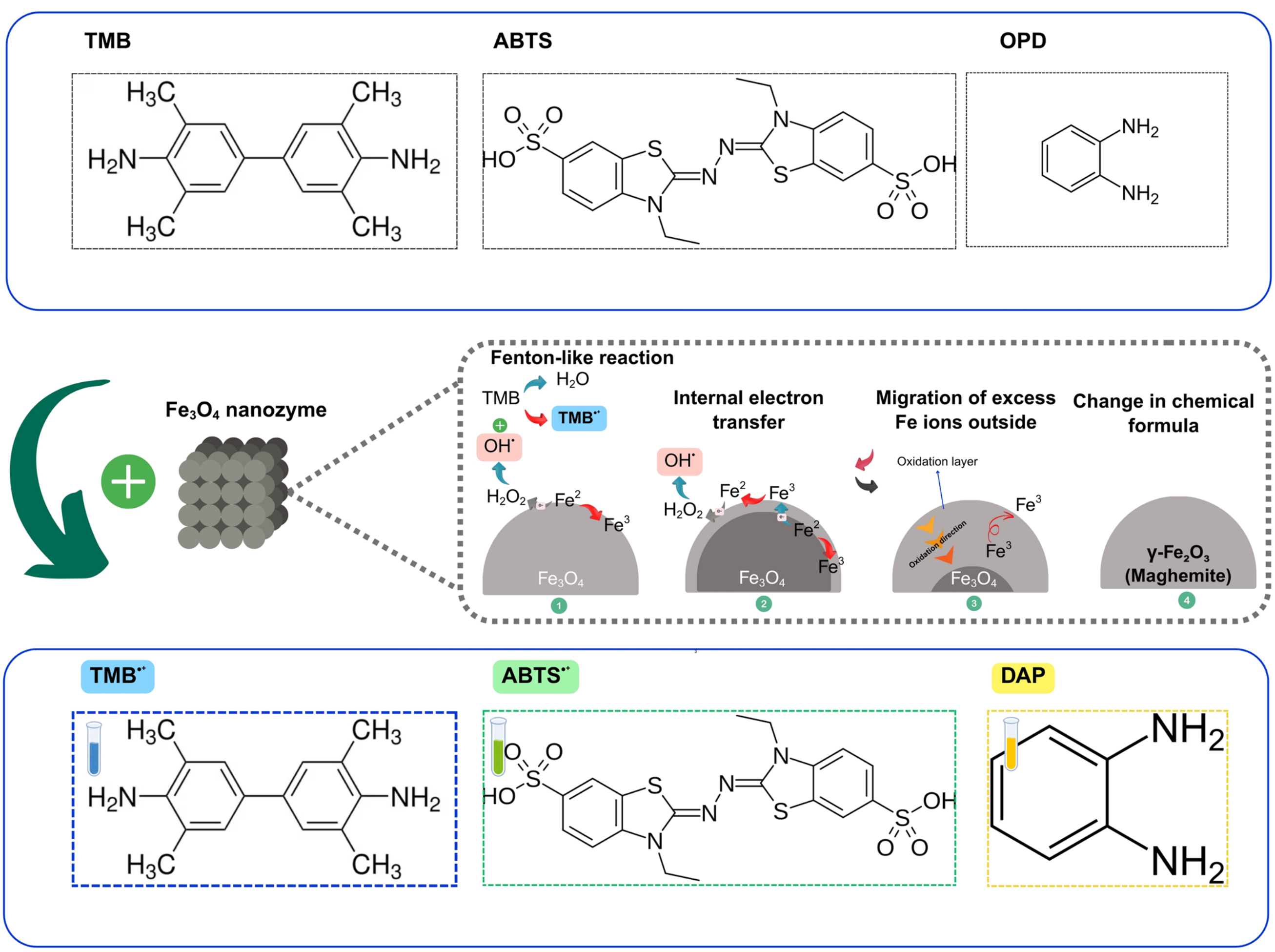

Fig. 5 Catalytic oxidation of TMB, ABTS, and OPD by Fe_3O_4 nanozymes with phase transformation insight. Created with Canva from https://www.canva.com

Additionally, the POD-mimicking efficiency of Fe_3O_4 NPs is regulated by various physicochemical and environmental factors, including particle sizes, surface area, shape, surface valency, crystallinity, states, pH, and metal doping or functionalization (Fig. 6). These parameters critically influence the availability of active sites and the redox cycling necessary for sustained catalytic activity (Guo et al. 2024; Wang et al. 2020a, b). Hence, a comprehensive understanding and control of these factors is essential for optimizing the catalytic performance of Fe_3O_4 nanozymes in diverse biomedical and environmental applications.

Size

Smaller Fe_3O_4 NPs generally demonstrate superior POD-like activity due to their higher surface-area-to-volume ratio, which provides more active sites for substrate binding and catalytic turnover rate (Wang et al. 2020a, b; Guo et al. 2024; Peng et al. 2008). This increased surface activity facilitates greater accessibility to H_2O_2 and chromogenic substrates such as TMB, thus accelerating the oxidation reaction rate. Additionally, the quantum size effect in ultrasmall NPs can influence surface electron distribution, promoting efficient Fe^{2+}/Fe^{3+} redox cycling, a core mechanism in Fenton-like catalysis (Lu et al. 2023).

Ai et al. (2023) demonstrated that ultrasmall high-entropy alloy nanoparticles (US-HEANPs, < 5 nm) displayed significantly enhanced catalytic and therapeutic performance. Their diminutive size not only improved catalytic efficiency by increasing active surface sites but also facilitated deeper tumor penetration and uniform biodistribution, key advantages for chemodynamic therapy. However, their synthesis remains challenging due to thermodynamic instability, underlining the importance of optimized fabrication techniques.

In a comparative study, Peng et al. (2008) evaluated Fe_3O_4 NPs of 11, 20, and 150 nm in size and observed a clear inverse correlation between particle size and POD-like activity. The enhanced efficiency of smaller particles was attributed to their higher specific surface area and increased number of reactive sites. Interestingly, Lu et al. (2023) reported that under acidic conditions (pH 4), larger Fe_3O_4 NPs (50 nm) displayed comparable or even higher catalytic activity when normalized to iron atom concentration. This was ascribed to their greater polycrystallinity and elevated Fe^{2+}/Fe^{3+} ratio, both of which support robust redox cycling. Moreover, dynamic light scattering (DLS) analysis revealed aggregation tendencies in both small and large NPs, potentially influencing their activity in solution. Notably, Fe_3O_4 nanozymes exhibited greater catalytic stability than natural peroxidase (HRP) and G-quadruplex (G4) DNAzyme in the presence of H_2O_2, maintaining sustained activity and structural integrity over time.

Structural morphology

The POD-like activity of Fe_3O_4 NPs is critically governed by their structural characteristics, including shape, crystallinity, and exposed crystal facets, which influence the accessibility and redox reactivity of surface iron atoms (Kang et al. 2020; Fu et al. 2017). Liu et al. (2011) investigated three morphological variants of IONPs, cluster spheres, triangular plates, and octahedra and reported that cluster spheres exhibited the highest catalytic activity, attributed to the prominent exposure of the (311) crystal plane, which harbors a greater density of catalytically active Fe^{2+}/Fe^{3+} sites. This reinforces

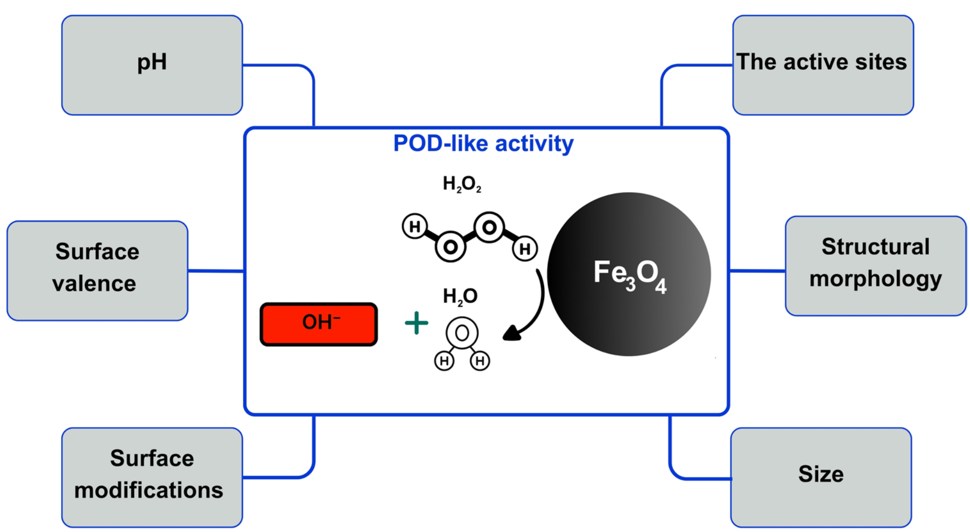

Fig. 6 Factors influence the POD-like activity of Fe_3O_4 nanozyme. Created with Canva from https://www.canva.com

the concept that specific morphologies can present more catalytically favorable surface terminations.

Puvvada et al. (2012) further demonstrated that truncated octahedral Fe_3O_4 NPs outperformed spherical ones due to exposure of high-energy facets such as (220), (111), and (100), with the (220) facet being particularly effective in presenting more accessible iron atoms for redox cycling. Similarly, Chaudhari et al. (2012) assessed the POD-like activity of α-Fe_2O_3 with hexagonal, cubic, and rod-like geometries, observing increased activity in the order: hexagonal < cubic < rod-shaped. Interestingly, among the rod-shaped NPs, the sample with the lowest surface area exhibited the highest catalytic activity, suggesting that other factors, such as facet orientation, electronic structure, or internal defects, may override surface area effects in controlling catalytic behavior.

Moreover, a study done by Gao et al. (2014) designed magnetically responsive Fe_3O_4/hydrogel nanozyme composites, where hydrogel concentration modulated particle morphology. At 10% hydrogel concentration, the resulting Fe_3O_4 nanozymes displayed uniform octahedral shapes with optimal catalytic performance. The inclusion of sulfonic acid groups (-SO_3^-) further enhanced iron ion binding and homogeneous particle distribution, underscoring how structural and chemical tuning synergistically enhances nanozyme activity. In conclusion, these findings highlight that nanozyme morphology, crystal facet exposure, and atomic arrangement are pivotal in dictating catalytic efficiency. Morphological engineering of Fe_3O_4 NPs can optimize the density and accessibility of surface-active iron species, thereby enhancing ROS generation and substrate oxidation. Therefore, precise structural control during synthesis is essential for designing next-generation nanozymes with tailored activity for biomedical and catalytic applications.

The active sites of Fe_3O_4 nanozyme compared to natural enzyme (HRP)

The POD-mimicking catalytic activity of Fe_3O_4 NPs primarily arises from surface Fe^{2+}/Fe^{3+} ions, which facilitate redox reactions like HRP (Wang et al. 2020a, b; Gao et al. 2017). However, despite having a comparable turnover number (*kcat*), Fe_3O_4 NPs exhibit significantly lower substrate affinity, reflected by their higher Michaelis–Menten constant (*Km*) compared to HRP (Gao et al. 2007; Fan et al. 2017). This discrepancy is largely attributed to the absence of the amino acid-rich active site found in HRP, particularly the histidine residues His42 and His170, which play essential roles in stabilizing H_2O_2 and promoting bond cleavage through hydrogen bonding (Fan et al. 2017).

To address this limitation, histidine-functionalized Fe_3O_4 NPs (His-Fe_3O_4 nanozymes) were developed to mimic the active site of HRP. This surface modification significantly improved H_2O_2 binding, reducing the *Km* value by an order of magnitude and increasing the overall catalytic efficiency ($kcat/Km$) by over 20-fold (Fan et al. 2017; Zhang et al. 2021a, b, c). The imidazole group in histidine enabled hydrogen bonding with H_2O_2, thereby enhancing both substrate recognition and catalytic turnover. However, this modification showed a limited effect on affinity for other substrates like TMB, likely due to electrostatic repulsion. In summary, these findings demonstrate that biomimetic modification of Fe_3O_4 nanozymes, by incorporating key functional groups from natural enzymes like HRP, can substantially enhance their catalytic performance.

Surface modifications

Surface modification plays a pivotal role in enhancing the POD-like activity of Fe_3O_4 NPs by modulating surface charge, catalytic microenvironment, and substrate accessibility. As catalytic activity predominantly occurs at the nanoparticle interface, engineering this surface through tailored functionalization strategies can markedly improve substrate affinity and catalytic efficiency (Tang et al. 2021). For instance, Liu et al. (2023a, b) developed a hybrid nanozyme (Fe_3O_4@MOF@Au) by sequentially coating Fe_3O_4 NPs with a metal-organic framework (MOF) followed by the in situ deposition of ultrasmall gold nanoparticles. This hierarchical surface design enhanced particle dispersion, promoted synergistic catalysis, and significantly increased hydroxyl radical (·OH) generation under H_2O_2 exposure, leading to improved antibacterial efficacy and accelerated wound healing.

In a separate study, Zha et al. (2022) employed polymer encapsulation to enhance nanozyme activity. Fe_3O_4 NPs were modified with dopamine and encapsulated in a polyacrylamide (PAM) nanogel. The amide groups in PAM facilitated interaction with persulfate ($Na_2S_2O_8$), enhancing sulfate radical (•SO_4^-) generation and achieving over 90% degradation of organic dyes, substantially outperforming uncoated Fe_3O_4 NPs. This highlights how polymer coatings can tune redox behavior and broaden environmental applications.

Further, small-molecule surface functionalization using amino acids has demonstrated promising therapeutic potential. Yuan et al. (2025a, b) functionalized Fe_3O_4 NPs with L-histidine, enabling coordination between its imidazole group and surface Fe ions. The resulting His-Fe_3O_4 nanozymes exhibited superior POD-like activity, potent antibacterial effects against *S. aureus* and *E. coli*, and enhanced wound healing performance, surpassing conventional antibiotics such as vancomycin.

Additionally, surface charge plays a critical role in substrate-specific catalytic activity. Coating Fe_3O_4 NPs with positively charged polymers like polylysine enhances interaction with negatively charged substrates (e.g., ABTS), while negatively charged coatings such as citrate or dextran facilitate oxidation of positively charged substrates like TMB (Tang et al. 2021). This electrostatic modulation allows for fine-tuning nanozyme activity based on target substrate properties. In summary, diverse surface engineering strategies, ranging from MOF/noble metal hybridization and polymer encapsulation to amino acid functionalization and charge modulation, are essential for optimizing Fe_3O_4 nanozymes. These approaches enhance their catalytic, antimicrobial, and environmental performance, making surface functionalization a cornerstone in the advancement of nanozyme technology.

Surface valence

The POD-like activity of Fe_3O_4 NPs is closely governed by the surface oxidation states of iron. Specifically, a higher Fe^{2+}/Fe^{3+} ratio enhances catalytic activity, as Fe^{2+} actively participates in redox reactions that mimic natural peroxidase enzymes (Gao et al. 2007; Zhang et al. 2021a, b, c). Chemical reduction strategies, such as $NaBH_4$ treatment, have been shown to increase the Fe^{2+} content on the Fe_3O_4 surface, thereby significantly improving the enzyme-mimetic behavior (Zhang et al. 2021a, b, c). This concept extends beyond Fe_3O_4 to other metal oxide nanozymes like cerium oxide and vanadium oxide, where surface valence tuning is likewise critical to catalytic performance (Yuan et al. 2021).

The zeta potential of nanoparticles is a useful parameter for modulating POD-like activity, since it influences the electrostatic adsorption of charged substrates and the colloidal stability of the catalyst. A study conducted by Gunatilake et al. (2024) reveals that the PB/PEI-NPs synthesized via a custom home-made vortex process exhibited a superior catalytic activity and a higher affinity for H_2O_2. This can be attributed to a synergistic combination of properties; for instance, their small particle size (19 ± 4 nm apparent and 23 nm crystalline) provided a high surface-to-volume ratio, which maximizes the number of active sites. And a high positive zeta potential ($+38.0 \pm 4.4$ mV) ensured excellent colloidal stability, preventing aggregation and maintaining surface accessibility.

Another recent research synthesized copper- and iron-containing phosphotungstates (Cu,Fe-PTs) as antibacterial nanozymes to combat Gram-negative bacteria. These nanozymes function by adhering to bacterial surfaces, increasing their interaction with H_2O_2, and weakening the antioxidant glutathione (GSH) of bacteria. The depletion, together with effective electron transfer via Fe^{2+}/Fe^{3+} and Cu^{2+}/Cu^{+} valence transitions, enhances the generation of ROS such as superoxide radicals ($\bullet O_2^-$) and •OH, which harm bacterial structures (Zhang et al. 2024a, b, c). Considering surface charge, research indicates that hexavalent chromium (Cr^{6+}, especially CrO_4^{2-}) may serve as a "switch" to boost the POD-like activity of silver nanoparticles (Ag NPs) on carbon spheres via a polyethylene glycol (PEG) linkage. It also revealed that Cr^{6+} changes the surface electronic structure of silver atoms and enhances reaction intermediate geometry. These adjustments lower free energy and modify bond lengths in the catalytic reaction, improving enzyme efficiency (Liu et al. 2022a, b).

pH

Another major factor influencing the POD-like activity of Fe_3O_4 NPs is pH. These nanozymes typically exhibit optimal activity under acidic conditions (pH 3.5–4.0), where they efficiently decompose H_2O_2 to generate hydroxyl radicals (•OH), essential for substrate oxidation (Zhang et al. 2021a, b, c). However, at physiological pH (~7.4), this activity diminishes, and Fe_3O_4 NPs tend to switch to catalase-like behavior, decomposing H_2O_2 into water and oxygen without ·OH formation, thus limiting their biomedical applicability (Zhang et al. 2021a, b, c). To address this limitation, researchers have developed functionalization strategies to maintain POD-like activity under neutral pH conditions. For instance, Vallabani et al. (2017) demonstrated that adenosine triphosphate (ATP) can modulate the local pH and create a favorable catalytic microenvironment around Fe_3O_4 NPs. ATP interacts with the nanoparticle surface, enhancing electron transfer processes and stabilizing intermediates, which leads to efficient ·OH production even at pH 7.4. This enabled effective glucose detection in human serum, highlighting the practical value of such pH-tuning strategies.

The proton-mediated breakdown of H_2O_2 is essential for POD-like activity. Protonation, especially of the distal oxygen, inhibits the O–O bond strength, promoting its heterolytic cleavage and allowing for significant electron transfer, in accordance with the proton-coupled electron transfer (PCET) processes noticed in natural peroxidases. Research indicates that protonation initiates the transformation from an inactive peroxide-bound complex (compound 0) to the catalytically active form, compound I. Similarly, in Fe_3O_4 nanozymes, acidic pH elevates proton availability, promoting H_2O_2 bond breakage and boosting POD-like activity (De Oliveira et al. 2021).

The applications of Iron oxide nanoparticles as peroxidase mimics

Fe_3O_4 NPs are gaining significant attention due to their exceptional magnetic, catalytic, and biocompatible characteristics, making them suitable for diverse applications across clinical, industrial, and environmental sectors (Guo et al. 2024). Their intrinsic POD-activity has positioned them as attractive nanozyme candidates, offering a viable alternative to natural enzymes. In the biomedical field, Fe_3O_4 nanozymes are extensively utilized for targeted drug delivery (Nordin et al. 2023) and magnetic resonance imaging (MRI) contrast enhancement (Liu et al. 2021). Environmentally, they are employed in pollutant degradation and removal (Chen et al. 2021a), while in analytical sciences, they serve as key components in nanozyme-based biosensors (Guo et al. 2024). These multifunctional roles underscore their versatility and broad applicability in modern technological and therapeutic platforms (Nguyen et al. 2021; Keum et al. 2023).

Colorimetric biosensor

Food safety remains a critical global concern, and colorimetric biosensing offers a promising solution due to its high sensitivity, visual readability, and operational simplicity. Among the various biosensors developed, Fe_3O_4 nanozyme-based platforms have garnered significant attention in this domain owing to their robust peroxidase-like activity, facile synthesis, tunable properties, and modifiable surfaces (Guo et al. 2024). These nanozymes catalyze the oxidation of H_2O_2, enabling the construction of low-cost and sensitive assays for reactive oxygen species (ROS) detection (Wei and Wang 2008). Their application extends beyond ROS sensing to include tumor marker detection, such as carcinoembryonic antigen (CEA) and alpha-fetoprotein (AFP), highlighting their utility in medical diagnostics (Zhuo et al. 2009).

Furthermore, Fe_3O_4 nanozymes have been applied in the sensitive detection of hazardous compounds such as pesticides and nerve agents, leveraging their catalytic stability and ease of integration (Guan et al. 2012; Liang et al. 2013; Yao et al. 2022). Notably, they have also been employed as catalytic probes in immunochromatographic test strips, enhancing the detection sensitivity of viral biomarkers (Duan et al. 2015). Altogether, these advancements underline the versatility and practicality of Fe_3O_4 nanozymes in biosensing technologies for food safety and public health monitoring.

Food composition analysis

Fe_3O_4 nanozymes have shown significant promise in food composition analysis due to their excellent peroxidase-like activity, ease of synthesis, and surface adaptability (Ju et al. 2023). Their application in colorimetric biosensors provides high sensitivity, low detection limits, and visual readouts, making them suitable for food safety monitoring. For instance, Niranjana et al. (2023) reported that rGO/Fe_3O_4 nanocomposites exhibit excellent electrochemical performance for detecting L-ascorbic acid (vitamin C) over a wide concentration range (10 µM to 100 mM), attributed to enhanced conductivity from Fe-rGO interactions. Similarly, Gaya et al. (2023) developed an Fe_3O_4-based electrochemical sensor capable of simultaneously detecting dopamine, uric acid, and ascorbic acid with high sensitivity and selectivity in complex matrices, showcasing its practical utility for multi-analyte food analysis.

Food safety analysis

Pathogenic bacteria, food additives, and antibiotic residues are critical food contaminants that pose significant health risks due to their potential to accumulate in the human body and cause diseases. Among food additives, L-cysteine is commonly used to prevent oxidative discoloration in products like fruit juices and fermented wheat items (Ju et al. 2023; Ma et al. 2023). Chen et al. (2018) synthesized one-dimensional Fe_3O_4 nanofibers with enhanced POD-like activity using a polymer-assisted thermochemical reduction technique. These nanofibers, with their increased surface area and redox activity, enabled highly sensitive colorimetric detection of L-cysteine through Fe^{3+} binding inhibition, which suppressed substrate oxidation and resulted in a measurable color change.

Regarding microbial contamination, foodborne pathogens such as *Staphylococcus aureus*, *Escherichia coli* O157:H7, *Salmonella enterica*, and *Listeria monocytogenes* are commonly implicated. Yu et al. (2024) developed a terahertz (THz) metamaterial biosensor functionalized with Fe_3O_4@Au nanocomposites and aptamers for the rapid detection of *S. aureus*. This platform allowed magnetic enrichment and significantly improved resonance signal Shifts in response to bacterial presence. The biosensor provided rapid detection in clinical Matrices Like spiked urine and blood, Matching traditional plate-counting accuracy but reducing detection time from 24 Hours to under 1 hour.

Additionally, Pang et al. (2019) introduced a surface-enhanced Raman spectroscopy (SERS)-based biosensor using Fe_3O_4@Au nanoparticles conjugated with both aptamers and vancomycin. This dual-recognition strategy enabled highly specific and sensitive identification of *S. aureus*, with successful performance in complex food samples. Together, these findings illustrate how Fe_3O_4-based nanocomposites offer powerful and rapid diagnostic solutions for monitoring food pollutants and pathogens, with significant potential for use in food safety and point-of-care diagnostics. Table 3 illustrates the Fe_3O_4-based nanozymes with their potentials in targeted sensing of foodborne contaminants.

Table 3 Fe_3O_4-based nanozymes applications in targeted sensing of foodborne contaminants

Nanozyme	Pathogenic bacteria	Sensitivity (LOD) (CFU/mL)	Ref
Fe_3O_4@Au nanozyme	*E. coli* O157:H7	2×10^2	Bazsefidpar et al. (2023)
Fe_3O_4 (Dap/Fe_3O_4)	*L. monocytogenes*	2.06	Li et al. (2025)
Fe_3O_4@Cu@PCPy yolk-shell nanozymes	*Salmonella enterica*	1.0	Hu et al. (2023)
Fe_3O_4@MOF@aptamer nanozyme	*E. coli* and *S. aureus*	$<10^2$	Lu et al. (2024)
Fe_3O_4@SiO_2@Ag nanozyme	*E. coli* and *S. aureus*	1.0	Mi et al. (2023)
Man-s Fe_3O_4 nanozyme	*Salmonella Typhimurium* and *Shigella Sp*	10^1 and 10^1,, respectively	Chen et al. (2021b)
Fe_3O_4@graphene nanozyme	*Salmonella*	2.4×10^2	Feng et al. (2022a, b)

POD-like activity of Fe_3O_4 NPs driven antibacterial strategies

Natural peroxidases, such as those produced by neutrophils, are essential components of the immune defense system, catalyzing the transformation of H_2O_2 into potent •OH that can effectively damage bacterial membranes, proteins, and genetic material. However, despite their functional importance, their practical use in clinical settings is hindered by factors such as high production costs, sensitivity to environmental conditions like pH and temperature, and challenging storage requirements (Ding et al. 2023).

As an alternative, Fe_3O_4 NPs have gained attention due to their inherent POD-like activity. These nanozymes offer several advantages, including low production cost, chemical durability, magnetic manipulability, and biocompatibility, making them suitable candidates for antibacterial strategies that do not rely on conventional antibiotics (Guo et al. 2022). Traditional antimicrobial agents, including antibiotics and certain inorganic substances, are increasingly limited by issues such as resistance development, environmental toxicity, and reduced reuse potential. In contrast, Fe_3O_4 nanozymes are characterized by long-term stability, broad-spectrum antibacterial action, and high catalytic performance, rendering them useful in the development of next-generation antimicrobial technologies (Shahraki et al. 2024).

Studies have demonstrated that Fe_3O_4 NPs exhibit dual functionality by combining their enzyme-mimicking and photothermal properties. Under mildly acidic conditions, they catalyze the decomposition of H_2O_2 into •OH radicals, leading to oxidative stress and bacterial cell death. Guo et al. (2022) showed that while Fe_3O_4 NPs alone had moderate effects on *E. coli* and *S. aureus*, their antibacterial activity was substantially enhanced in the presence of H_2O_2. When coupled with near-infrared (NIR) irradiation, their bactericidal efficiency significantly increased, especially at higher concentrations (e.g., 1000 μg. mL^{-1} with both H_2O_2 and NIR), resulting in near-complete bacterial elimination.

To improve performance beyond what unmodified Fe_3O_4 NPs can achieve, composite nanozymes have been developed. For instance, Liu et al. (2023a, b) engineered a multifunctional hybrid nanozyme composed of Fe_3O_4 stabilized with a metal-organic framework (MOF) and decorated with ultrasmall gold nanoparticles (Fe_3O_4@MOF@Au, or FMA NPs). This construct demonstrated enhanced catalytic efficiency at low H_2O_2 concentrations, potent antibacterial action against *E. coli* and *S. aureus*, and excellent compatibility with biological tissues. In vivo assessments confirmed its capacity to promote wound healing and manage bacterial infections without the use of antibiotics. In conclusion, Fe_3O_4 nanozymes represent a robust and effective substitute for natural peroxidases in antibacterial applications. Their catalytic resilience, ease of synthesis, and tunable functionality make them promising tools in the fight against antibiotic-resistant pathogens. Innovations such as surface modification and multimodal therapy have further advanced their potential in biomedical applications, particularly for wound care and infection control.

Cancer therapy

Fe_3O_4 nanozymes are gaining increasing attention in cancer therapy due to their inherent POD-like activity and compatibility with tumor microenvironments. These nanozymes catalyze the decomposition of H_2O_2 into highly reactive ·OH, inducing oxidative stress and promoting apoptosis in cancer cells through Fenton-like mechanisms (Moradi Hasan-Abad et al. 2024). Interestingly, PtFe@Fe_3O_4 nanozymes developed by Li et al. (2021) successfully merged photothermal therapy and catalytic ROS generation, showing intensified therapeutic performance and improved oxygenation in hypoxic tumor sites under light activation.

Further supporting this approach, Aslan (2025) employed a green synthesis method using *Polygonum cognatum* extract to fabricate ultrasmall Fe_3O_4 NPs (~3.1 ± 1.2 nm). These nanozymes exhibited robust POD and catalase-like activity, as well as effective ROS generation. In cellular assays, they induced 80.08% apoptosis in MDA-MB-231 breast cancer cells, with significantly lower toxicity (22.63%) toward normal MCF-10A cells, indicating selective cytotoxicity driven by oxidative stress. In another approach, Du et al. (2020) synthesized hollow Fe_3O_4 mesocrystals capable of simultaneously enhancing magnetic hyperthermia and chemodynamic therapy. Their efficacy was attributed to a high Fe^{2+}/Fe^{3+} ratio and abundant oxygen vacancies, which amplified both thermal conversion and ROS production. This dual functionality allowed for efficient, synergistic cancer treatment with a single material platform.

Interestingly, the morphology of Fe_3O_4 nanozymes also plays a critical role in their therapeutic activity. Fu et al. (2017) demonstrated that nanoclusters outperformed nanodiamonds and nanoflowers in terms of catalytic efficiency. When combined with low doses of H_2O_2, these nanostructures promoted cancer cell death through ROS-mediated mechanisms. However, the study also noted that in vitro enzymatic activity did not always correlate with cytotoxic efficacy, emphasizing that cellular uptake and intracellular processing are equally important in determining overall therapeutic outcomes.

Assessing the cytotoxicity and concentration thresholds of Fe_3O_4 nanozyme for biomedical use

Numerous studies have assessed how factors like particle size, surface modifications, and dosage levels affect their cytotoxic profile. Smaller Fe_3O_4 NPs typically demonstrate higher cellular uptake and greater surface interaction potential, which can increase biological effects (Babaei and Ganjalikhani 2014; Anuje et al. 2021). However, the impact of their physicochemical properties, including shape, size, and surface chemistry, must be carefully optimized to mitigate cytotoxic risks (Montiel Schneider et al. 2022; Vargas et al. 2005).

Despite their advantageous traits such as magnetic responsiveness, surface tunability, and potential in radiosensitization, Fe_3O_4 NPs are known to induce ROS, including $\cdot OH$, O_2^-, H_2O_2, and $HO_2\cdot$, via Fe^{2+} interactions, contributing to oxidative stress and dose-dependent cytotoxicity (Klein et al. 2012). For instance, da Silva Bruckmann et al. (2020) studied the cytotoxicity of SiO_2-coated Fe_3O_4 nanocomposites in L-929 cells using MTT and neutral red assays. Their results indicated that doses ≤ 50 µg. mL^{-1} were relatively biocompatible, Maintaining cell viability above 70%, while exposure to 500 µg. mL^{-1} significantly reduced viability (~46%), emphasizing the need for concentration limits in biomedical use.

A recent study by Hasan et al. (2025) identified the safest concentration of Fe_3O_4 NPs (0.003 mg. mL^{-1}) synthesized by *Bacillus proteolyticus* UPMC1508 for potential biomedical applications. However, further investigation is required to evaluate cytotoxic effects across a broader concentration range.

Additionally, surface modification strategies for uncoated Fe_3O_4-NPs remain underexplored, which could be key to enhancing their biocompatibility and functional performance in clinical settings. Surface engineering has shown promise in improving Fe_3O_4 biocompatibility. Anuje et al. (2021) found that uncoated Fe_3O_4 NPs significantly reduced cell viability at concentrations ≥ 0.1 mg. mL^{-1}, whereas surface coatings such as polyethylene glycol (PEG), silica, and Schiff base ligands improved tolerance. For example, PEG-coated Fe_3O_4 maintained ~45% viability at 0.25 mg. mL^{-1}, and silica-based formulations showed Minimal toxicity up to 0.128 µg. mL^{-1}. Additionally, functionalizing Fe_3O_4 with bioactive compounds like quercetin allowed for selective cytotoxicity against cancer cells while preserving normal fibroblast viability.

Supporting this, Asl et al. (2024) synthesized a gelatin-starch-based Fe_3O_4 nanocarrier loaded with quercetin (G/S/Fe_3O_4@Quercetin), showing ~43.5% apoptosis induction in HepG2 liver cancer cells but negligible toxicity toward L-929 fibroblasts at equivalent doses. These results highlight the therapeutic selectivity and biosafety of well-designed nanoformulations. Overall, Fe_3O_4 NPs demonstrate promising potential for biomedical use, but their safety is highly dependent on concentration and surface properties. The consensus across studies suggests that safe working concentrations typically Lie between 0.01 and 0.1 µg. mL^{-1}, especially for applications involving L-929 cells, reinforcing the importance of tailored design and dosing in clinical translation.

While the cytotoxicity of bare Fe_3O_4 NPs has been extensively studied, there remains a significant gap in evaluating the safety profile of Fe_3O_4 NPs across varying concentrations and also assessing the cytotoxicity of POD-like Fe_3O_4 nanozyme. Only a few studies, such as those involving L-histidine functionalized Fe_3O_4 nanozymes and Fe_3O_4@MOF@Au hybrids, have reported encouraging biosafety outcomes in vitro and in vivo (Yuan et al. 2025a, b; Liu et al. 2023a, b). Yuan et al. (2025a, b) demonstrated that L-histidine-functionalized Fe_3O_4 nanozymes (after 7 days of treatment) exhibited excellent antibacterial activity and in vivo wound healing efficacy without causing toxicity to normal tissues, though specific in vitro concentrations were up to 500 µg. mL^{-1}. Similarly, Liu et al. (2023a, b) reported that the hybrid nanozymes (Fe_3O_4@MOF@Au, FMA NPs) exhibited outstanding biosafety, as cell viability in HUVECs and L-02 hepatocytes remained above 95% at low concentrations and over 90% even at a high dose of 400 µg. mL^{-1} after 24 h of exposure. These findings confirm the excellent biocompatibility and negligible cytotoxicity of FMA NPs. However, broader toxicological evaluations across various nanozyme formulations and biological models are still lacking. This limitation highlights the need for further comprehensive studies to validate the clinical translation and safe application of Fe_3O_4 nanozymes.

Conclusion

The biosynthesis of Fe_3O_4 NPs by bacteria offers a sustainable and advantageous alternative to traditional chemical synthesis techniques. This sustainable method provides several benefits, such as diminished environmental toxicity, decreased energy usage, economic efficiency, and the integration of biologically sourced stabilizers that improve nanoparticle biocompatibility and functional stability. These biosynthetic approaches conform to the tenets of green chemistry and are particularly beneficial for biomedical and environmental applications. Fe_3O_4 NPs have emerged as versatile and effective substitutes for natural enzymes, offering notable advantages such as biocompatibility, magnetic responsiveness, and enzyme-mimicking catalytic functions. These properties support a broad range of applications, from antibacterial and anticancer therapies to biosensing and environmental remediation. Advances in surface functionalization, hybrid nanozyme construction, and tailored synthesis have greatly improved their stability and performance across different operating environments. However, several challenges must still be addressed. Their catalytic efficiency can decline under physiological pH or prolonged exposure due to phase changes, and their activity is highly dependent on factors such as particle size, morphology, and surface chemistry. A significant limitation lies in the lack of studies assessing the cytotoxicity and biosafety of Fe_3O_4 NPs specifically at wider concentrations, and those with POD-like activity. This represents a crucial research gap. To fully realize the clinical and industrial potential of Fe_3O_4 NPs, future efforts should prioritize in-depth mechanistic studies, standardized biocompatibility assessments, and long-term safety evaluations. Moreover, addressing issues related to scale-up and regulatory compliance will be vital for translating these nanozymes from laboratory innovation to practical applications.

Author contribution YRH conceptualized and designed the review, conducted the literature search, synthesized and analyzed the collected information, and prepared the initial manuscript draft. RM supervised the overall development of the manuscript, offering guidance on structure, scope, and critical content evaluation. FWFW, SEA, and MH contributed through regular discussions, provided feedback on the scientific interpretation, and supported the refinement of the manuscript. All authors reviewed and approved the final version of the manuscript.

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Declarations

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