Chapter 9
Nanozymes: Biomedical Applications of Enzymatic Fe_3O_4 Nanoparticles from *In Vitro* to *In Vivo*

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Abstract Fe_3O_4, also called magnetite, is a naturally occurring mineral and has been widely used in biomedical applications. However, in the past, all the applications were based on its excellent magnetic properties and neglected its catalytic properties. In 2007, we found that Fe_3O_4 nanoparticles are able to perform intrinsic enzyme-like activities. A specific term, "nanozyme", is used to describe the new property of intrinsic enzymatic activity of nanomaterials. Since then, Fe_3O_4 nanoparticles have been used as enzyme mimics, which broadens their applications beyond simply their magnetic properties, with applications in biomedical diagnosis and therapy, environmental monitoring and treatment, the food industry and chemical synthesis. In this chapter, we will summarize the basic features of Fe_3O_4 as an enzyme mimetic and its applications in biomedicine.

Keywords Nanozymes · Enzyme-like activity · Enzyme mimetic · Fe_3O_4 · Biomedical application

Abbreviations

ABTS	2, 2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid)
AD	Alzheimer's disease
APTES	3-Aminopropyltriethoxysilane
CNT	Carbon nanotube
DAB	3, 3′-Diaminobenzidine

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EBOV	Ebola virus
EPR	Enhanced permeability and retention
Fe_3O_4	Magnetite or iron oxide
GO	Graphene oxide
GOx	Glucose oxidase
H_2O_2	Hydrogen peroxide
HCG	Human chorionic gonadotropin
HFn	Human heavy-chain ferritin
HRP	Horseradish peroxidase
M-HFn	Magnetoferritin nanoparticles
MNPs	Magnetic nanoparticles
MRI	Magnetic resonance imaging
MRSA	Staphylococcus aureus
NPs	Nanoparticles
NTs	Nanotubes
NWs	Nanowires
OPD	o-phenylenediamine
PD	Parkinson's disease
PEG	Polyethylene glycol
RES	Reticuloendothelial system
RGO	Reduced graphene oxide
ROS	Reactive oxygen species
TMB	3, 3′, 5, 5′-Tetramethylbenzidine

9.1 Introduction

Iron (II, III) oxide (Fe_3O_4) is the naturally occurring mineral magnetite, which is a black powder with permanent magnetism. However, this material exhibits special superparamagnetic magnetization when the size is reduced to the nanoscale (e.g. 1–100 nm) [1, 2], so that Fe_3O_4 nanoparticles can be easily aggregated in the presence of an external magnetic field and can be rapidly re-dispersed with the removal of the magnetic field. Based on this unique property, Fe_3O_4 nanoparticles have been applied in various fields, particularly in biomedicine, including bio-separation and purification, biosensors, transfection, MRI, hyperthermia therapy, targeted drug delivery and as a theranostic platform (Fig. 9.1) [3–8].

However, nanoscale Fe_3O_4 has many specific physical and chemical properties besides magnetism, including hyperthermia, photothermal properties, photoacoustic effects, and fluorescence [9]. Its recently reported enzyme-like catalytic properties are of particular interest. In 2007, Fe_3O_4 nanoparticles were discovered to possess intrinsic peroxidase-like activity, similar to that of horseradish peroxidase (HRP) [10]. Since then, many nanomaterials have been found to possess enzymatic activities, which dramatically broadens their applications in biomedicine. The term "nanozyme" [11] was introduced to describe the phenomenon of nanomaterials with intrinsic enzyme-like activities [12–15], with Fe_3O_4 nanoparticles being the first reported example of a nanozyme. In this chapter, we mainly focus on the enzymatic

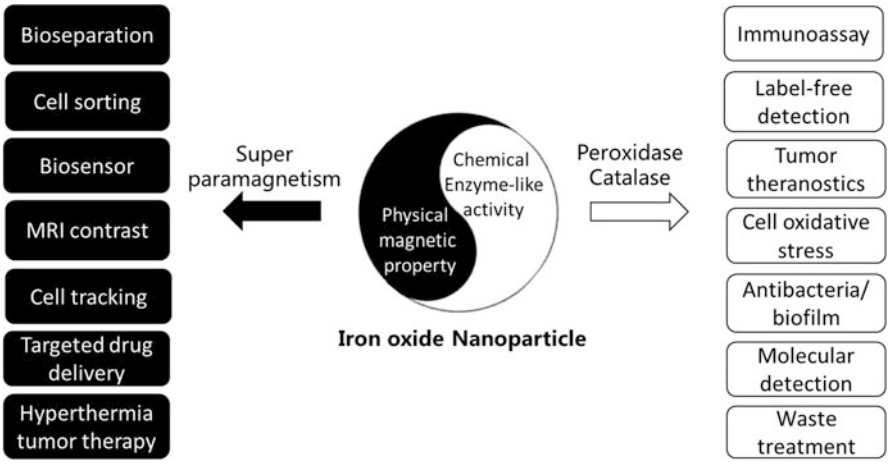

Fig. 9.1 Iron oxide nanoparticles with intrinsic enzyme-like activity allow novel applications in addition to those based on their magnetic properties

properties of Fe_3O_4 nanoparticles and summarize the novel applications of this nanozyme, from *in vitro* bioassays to *in vivo* diagnosis and therapy (Fig. 9.1).

9.2 Basic Features of Fe_3O_4 Nanozymes

As enzyme mimetics, Fe_3O_4 nanozymes show similar catalytic properties to natural enzymes, including substrate specificity, pH and temperature optima, kinetics and mechanism. In general, they are typical nanomaterials which have a diameter on the nanoscale i.e. 1–500 nm and specific nanocrystalline structures, morphologies and surface modifications, providing nanozymes with many advantages over natural enzymes because they are robust inorganic nanomaterials. Therefore, Fe_3O_4 nanozymes have great potential for use as multi-functional nanomaterials for biomedical applications.

9.2.1 Activities of Fe_3O_4 Nanozymes

(1) **Peroxidase-like activity**: Fe_3O_4 nanozymes were first found to have peroxidase-like activity, catalyzing the reaction of H_2O_2 with chromogenic reagents such as TMB, OPD, DAB and ABTS (Fig. 9.2a–c). The optimal conditions for the catalysis are similar to those for HRP i.e. 37–40 C under acidic pH (e.g. sodium acetate buffer pH 3–6.5) which favors the generation of free radicals to oxidize TMB (Fig. 9.2d).
(2) **Catalase-like activity**: Fe_3O_4 nanozymes also show catalase-like activity, decomposing H_2O_2 into oxygen and water under neutral/basic pH conditions

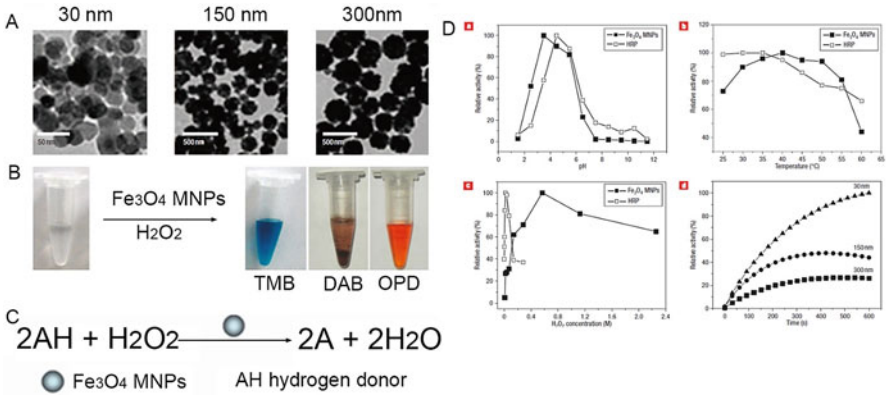

Fig. 9.2 Fe_3O_4 nanoparticles show intrinsic peroxidase-like activity [10]. (**a–c**) Different sizes of Fe_3O_4 nanozymes and colorimetric reactions catalyzed by Fe_3O_4 nanozymes. (**d**) Reaction conditions for Fe_3O_4 nanozymes (300 nm) (*a–c*) and comparison of the activity of Fe_3O_4 nanozymes with different sizes (*d*). (Reprinted from Ref. [10] with permission from the Nature Publishing Group)

Table 9.1 Typical parameters for Michaelis-Menton kinetics [10]

	[E] (M)	Substrate	K_M (mM)	V_{max} (s^{-1})	k_{cat} (M^{-1} s^{-1})
Fe_3O_4 MNPs	9.402×10^{-13}	TMB	0.098	0.0032	3.37×10^9
Fe_3O_4 MNPs	9.402×10^{-13}	H_2O_2	154	0.0091	9.68×10^9
HRP	2.5×10^{-11}	TMB	0.434	0.0093	3.72×10^8
HRP	2.5×10^{-11}	H_2O_2	3.70	0.0081	3.24×10^8

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[16]. Fe_3O_4 nanozymes can decompose H_2O_2 into oxygen and water by this enzymatic activity, which may be used to scavenge H_2O_2 in biosystems.

9.2.2 *Kinetics and Mechanism of Fe_3O_4 Nanozymes*

The catalysis of Fe_3O_4 nanozymes follow Michaelis-Menten kinetics. Curves for both H_2O_2 and TMB in peroxidase-like catalysis fit the equation $v = (V_{max}[S])/(K_M + [S])$, where V_{max} represents the maximum velocity, [S] is the substrate concentration, and K_M is the Michaelis constant (Fig. 9.3). The related parameters are shown Table 9.1. The k_{cat} is equal to V_{max}/[E]. The K_M value of H_2O_2 for Fe_3O_4 nanozymes is higher than that for HRP, indicating lower affinity of H_2O_2 for Fe_3O_4 nanozymes. In contrast, the K_M value of TMB for Fe_3O_4 nanozymes is lower than that for HRP, indicating that Fe_3O_4 nanozymes have higher affinity for TMB. Notably, a single Fe_3O_4 nanozyme with 300 nm diameter showed 40 times higher activity than a single HRP molecule.

Just like HRP, the catalysis of Fe_3O_4 nanozymes follows a ping-pong mechanism. In the peroxidase-like catalysis, a set of parallel lines are produced when a set of v against [S] (fixed H_2O_2, varying TMB or fixed TMB, varying H_2O_2) are

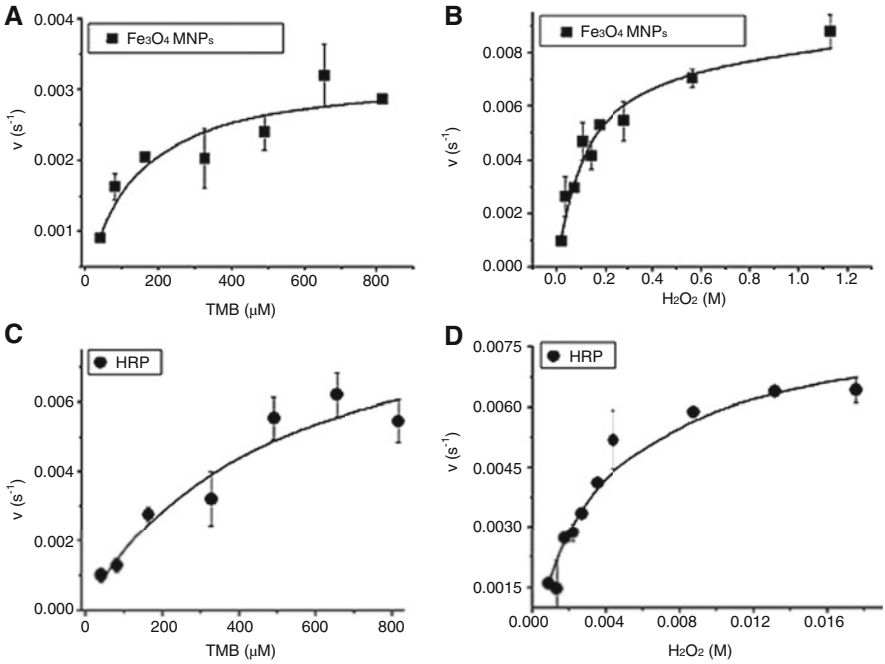

Fig. 9.3 Apparent Michaelis-Menton kinetics for Fe_3O_4 nanozymes in comparison with HRP. (Reprinted from Ref. [10] with permission from the Nature Publishing Group)

plotted in a Lineweaver-Burk plot. That is, H_2O_2 first binds to and react with Fe_3O_4 nanozymes, releasing the first product, then TMB reacts with Fe_3O_4 nanozymes.

It is important to note that the peroxidase activity is not derived from free iron ions via the Fenton reaction. The trace amount of iron released from the surface of Fe_3O_4 nanozymes was around two orders of magnitude lower than the concentration required for the Fenton reaction, only showing negligible catalytic activity [17–20]. These results demonstrate that the observed reaction cannot be attributed to leaching of iron ions into solution, but occurs on the surface of the nanozymes. Importantly, ferrous iron (Fe^{2+}) seems to be more important than ferric iron (Fe^{3+}) in the enzyme-mimicking catalysis, as increasing the ratio of Fe^{2+} in Fe_3O_4 nanoparticles enhances the peroxidase-like activity [10]. Taken together, the intrinsic enzyme-like property of Fe_3O_4 nanozymes arises from the variable valence of iron in the nanoparticles.

9.2.3 Advantages of Fe_3O_4 Nanozymes

Compared to natural enzymes, nanozymes show many advantageous features. Fe_3O_4 nanozymes show enhanced stability towards extreme conditions such as temperature (4–90 °C) and pH (2–12) [21]. In contrast, the enzyme HRP did not show any activity after treatment at pH lower than 5 and lost activity rapidly when

the temperature was greater than 40 °C. Fe_3O_4 nanoparticles can be stored long term and reused many times [22–26]. In addition, the activity of Fe_3O_4 nanozymes can be tuned by modulating the size, structure or morphology [18, 27–30] (Fig. 9.4), by the addition of dopants [31–34], by surface modifications [35–38] or by hybridizing with other nanomaterials [19, 23, 39–44], which allows the design of nanozymes with optimized activity for the specific purpose required.

Further, Fe_3O_4 nanozymes have both magnetic and catalytic properties and so can simultaneously perform two basic functions: enzyme-like activity and superparamagnetism. Regarding the enzyme-like activity, Fe_3O_4 nanozymes, as mentioned above, can mimic peroxidase and catalase activities [16]. Fe_3O_4 nanozymes can also be used as a carrier to conjugate other functional molecules or groups to construct a cascade reaction [17]. Finally, Fe_3O_4 nanozymes can be synthesized by chemical methods such as solvothermal reaction, sol-gel and co-precipitation. The required chemical reagents are usually much cheaper than biological reagents. Scale up of production is therefore both straightforward and economical. The multifunctionality of Fe_3O_4 nanozymes facilitates a wide range of practical applications, including in biomedicine, such as tumor theranostics, ultrasensitive molecular detection and controlled drug release. The combination of these functions can be used to create versatile state-of-the-art technologies and strategies.

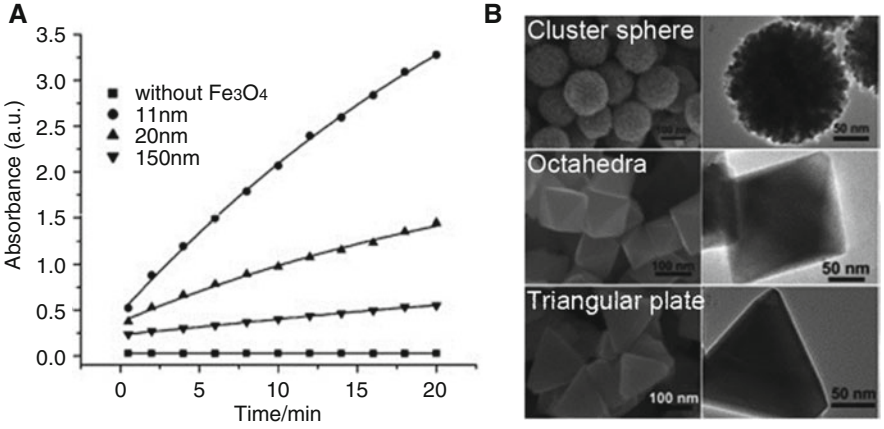

Fig. 9.4 (**a**) Tuning of activity by variation of size. Reprinted from Ref. [28] with permission from Elsevier. (**b**) Tuning of activity by variation of morphology (peroxidase-like activity: cluster spheres > triangular plates > octahedral). (Reprinted from Ref. [18] with permission from John Wiley & Sons Ltd.)

9.3 Biomedical Applications of Fe_3O_4 Nanozymes

The discovery of enzymatic activity of Fe_3O_4 nanozymes has stimulated their use in a range of applications, including immunoassays, tumor diagnosis and therapy, biosensors, antibacterial/antibiofilm reagents, environmental monitoring and pollutant degradation, in the food industry and in chemical synthesis. Here we summarize their state-of-the-art applications particularly in the biomedical field, which span *in vitro* molecular detection to *in vivo* diagnosis and therapy of tumors and diseases.

9.3.1 In Vitro Bioassays

The peroxidase-like activity of Fe_3O_4 nanozymes make them an ideal alternative to HRP and thus they can be used to replace HRP in enzyme-linked immunosorbent assay (ELISA) and HRP-related molecular detection [45, 46]. Fe_3O_4 nanozymes [47] can be conjugated with an antibody and applied in ELISA to amplify signals by catalyzing a colorimetric reaction (Fig. 9.5a). Importantly, due to their magnetism, Fe_3O_4 nanozymes with an appropriate ligand or antibody can be employed to capture and enrich very low amounts of sample, which will improve the sensitivity and efficiency of detection [48]. According to this principle, a capture-detection immunoassay has been developed to detect carcinoembryonic antigen (CEA) with a detection limit up to 1 ng mL^{-1} [45]. This kind of nanozyme-based immunoassay can be used to detect multiple antigens including biomarkers and bacteria or cells, such as IgG, hepatocellular carcinoma biomarker GP73 [49], human chorionic gonadotropin (HCG) [50], *Mycoplasma pneumoniae* [51], *Vibrio cholerae*, rotavirus [52], and cancer cells with human epidermal growth factor receptor 2 (HER2) [52, 53].

A nanozyme-strip has been developed by combining the magnetism and peroxidase-like activity of Fe_3O_4 magnetic nanoparticles. This novel strip can be used to detect the glycoprotein of Ebola virus (EBOV) as low as 1 ng/mL, showing a 100-fold increased sensitivity compared to the classic gold-strip method [55] (Fig. 9.6b). Importantly, the nanozyme-strip shows comparable sensitivity and accuracy with ELISA in the detection of EBOV and New Bunyavirus clinical samples. In addition, the nanozyme-strip is much faster (within 30 min) and simpler (observed by naked eye) than ELISA. These results indicate that the nanozyme-strip may be used as a point-of-care test for EBOV detection, providing a simple method for diagnosis of infection in Ebola-affected areas of Africa.

Besides immunoassays via antibody-antigen recognition, other detection methods have been developed based on the specific interaction between DNA molecules or aptamers. Park and coworkers developed a label-free colorimetric detection method for nucleic acids by comparing catalytic activity before and after DNA binding on Fe_3O_4 nanozymes [56]. Thiramanas et al. [57] developed a novel and

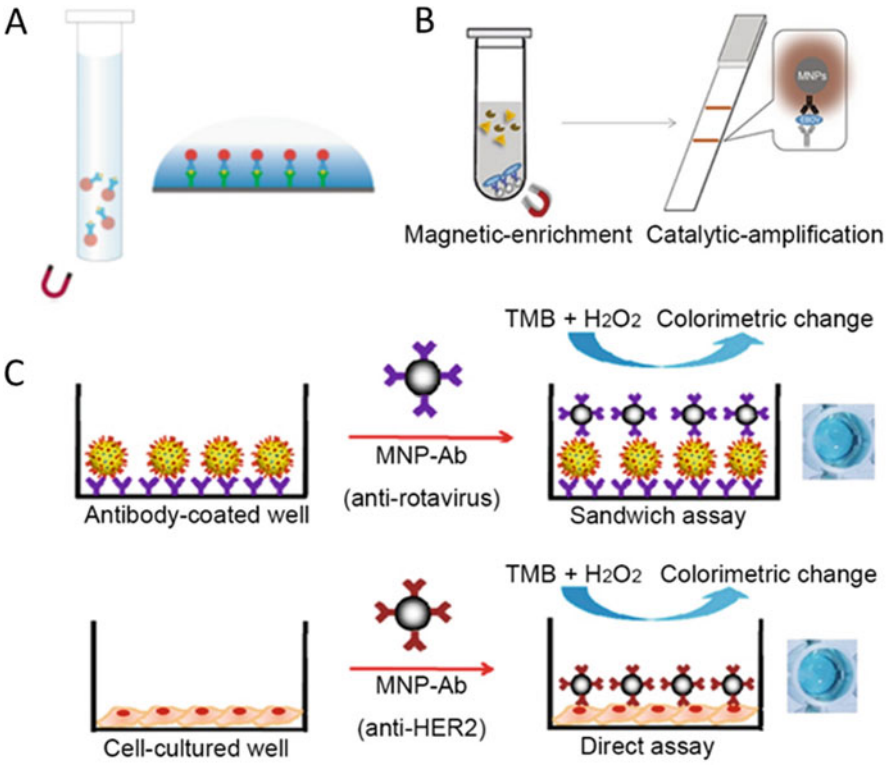

Fig. 9.5 Novel immunoassays based on Fe_3O_4 nanozymes. (**a**) Traditional immunoassay. Reprinted from Ref. [54] with permission from the Nature Publishing Group. (**b**) Nanozyme-strip for Ebola detection. Reprinted from Ref. [55] with permission from Elsevier. (**c**) Virus and cancer cell detection. (Reprinted from Ref. [52] with permission from MDPI AG)

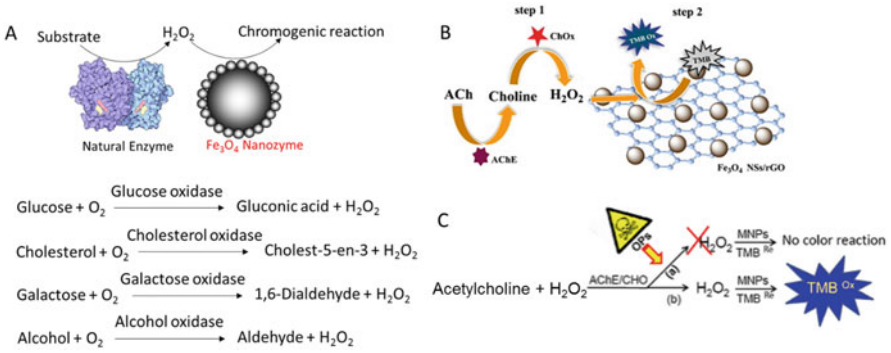

Fig. 9.6 Cascade reactions for molecular detection. (**a**) Dual sequential reactions. (**b** and **c**). Triple sequential reactions for acetylcholine (Ach) [24] and pesticide detection [73]. (Reprinted from Ref. [24, 73] with permission from Elsevier and the American Chemical Society, respectively)

sensitive system for *Vibrio cholerae* detection using magnetic polymeric nanoparticles (MPNPs) composed of a Fe_3O_4 core with peroxidase activity in combination with polymerase chain reaction to integrate magneto–PCR–colorimetry in one system. Aptamers also can be used in similar ways as they can specifically recognize target molecules with high affinity.

Finally, it is feasible to develop cascade catalytic reactions by conjugating natural enzymes onto Fe_3O_4 nanozymes. In a cascade reaction, natural enzyme is responsible to generate H_2O_2 and then Fe_3O_4 nanozymes utilize H_2O_2 to generate colorimetric signals. Wei and Wang [17] first developed a cascade system by conjugating glucose oxidase (GOx) onto Fe_3O_4 nanozymes for glucose detection (Fig. 9.6a). In this hybrid system, GOx catalyzes glucose to generate H_2O_2 and Fe_3O_4 nanozymes then uses H_2O_2 as substrate to produce a colorimetric signal. There is a directly proportional relationship between glucose concentration and the colorimetric signal [17]. In this way, a glucose-response curve can be established with a detection limit for glucose as low as 3×10^{-5} mol L^{-1} and a linear range from 5×10^{-5} to 1×10^{-3} mol L^{-1}. Since then, many groups have used this method by combining GOx with or integrating GOx onto Fe_3O_4 nanozymes for glucose detection [21, 41, 43, 58–72], showing great potential in measuring blood glucose levels for diabetes diagnosis.

Alternatively, other oxidases can be integrated into Fe_3O_4 nanozymes to detect substrates besides glucose, including cholesterol [71], galactose [74], alcohol [75], and acetylcholine (ACh) [24, 73] (Fig. 9.6b, c).

9.3.2 Ex Vivo Tracking and Histochemistry Diagnosis

Iron oxide nanoparticles are often used as diagnostic and therapeutic agents for biomedical applications due to their superparamagnetism. Quantitative analysis of their biodistribution, pharmacokinetics and organ clearance in animal models is important to understand their *in vivo* behavior and biosafety. A novel histochemical method for visualizing unlabeled Fe_3O_4 NPs in mouse tissues was developed by our group, which employs the intrinsic peroxidase activity of the NPs to produce a color reaction [76] (Fig. 9.7). It was found that dextran-coated Fe_3O_4 NPs were mainly localized in the liver, spleen and lung rather than the kidney, lymph nodes or thymus. Cellular location was further examined by combining hematoxylin-eosin (H&E) staining. Dextran-coated Fe_3O_4 NPs were taken up mainly by the reticuloendothelial system (RES) in these organs, including Kuppfer macrophage cells in the liver, alveolar macrophages in the lung and macrophage perifollicular areas in the spleen.

Organ clearance could also be evaluated in the same way, showing that the content in liver, spleen and lung increased steadily between 0.25 and 5 h post-injection, and then rapid clearance occurred between 5 and 72 h post-injection. This approach is more sensitive when compared with the traditional Prussian blue staining method because of the highly effective catalytic activity of Fe_3O_4 NPs. Importantly, without

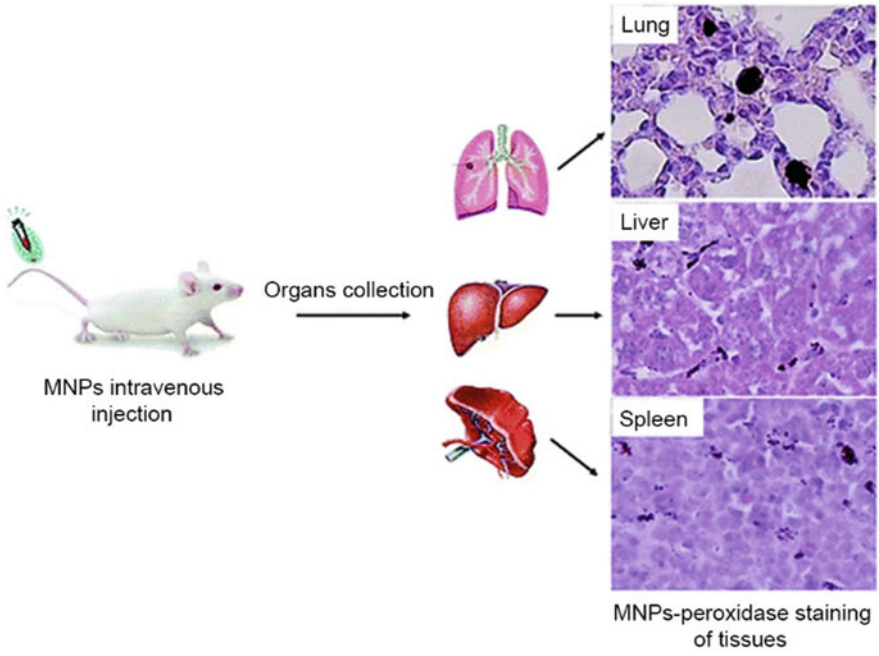

Fig. 9.7 Label-free detection of Fe_3O_4 NP distribution via their peroxidase activity [76]. (Reprinted from Ref. [76] with permission from the American Chemical Society)

labeling with exogenous indicators, it could reduce false signals from background and may provide a better way to understand the real behavior of NPs *in vivo* and thus has significant implications for the clinical translation of Fe_3O_4 NPs. Presumably, other nanoparticles having intrinsic peroxidase activity could also be studied in a similar way to determine their *in vivo* behavior.

Fe_3O_4 nanozymes also can be used for histochemistry diagnosis by integrating with a specific antibody or protein. In particular, Fe_3O_4 nanozymes can be assembled into ferritin to form recombinant magneto ferritin nanoparticles (M-HFn) [77] achieving the capability of tumor targeting and visualization in the same unique system. Fe_3O_4 nanozymes are encapsulated inside and the recombinant human heavy-chain ferritin (HFn) proteins form a protein shell which can recognize tumor cells overexpressing transferrin receptor (TfR1) (Fig. 9.8). Once bound to tumor tissues, the Fe_3O_4 core performs peroxidase-like activity to conduct a colorimetric reaction in the presence of H_2O_2 and then the tumor tissues can be visualized. In this way, 474 clinical specimens from patients with nine types of cancers were examined in a single-step binding and colorimetric reaction. Fe_3O_4 nanozymes successfully distinguished cancerous cells from normal cells with a sensitivity of 98% and specificity of 95%. These results demonstrate that Ferritin- Fe_3O_4 nanozymes can be used as a diagnostic reagent for rapid, low-cost and universal assessment of malignant tumors.

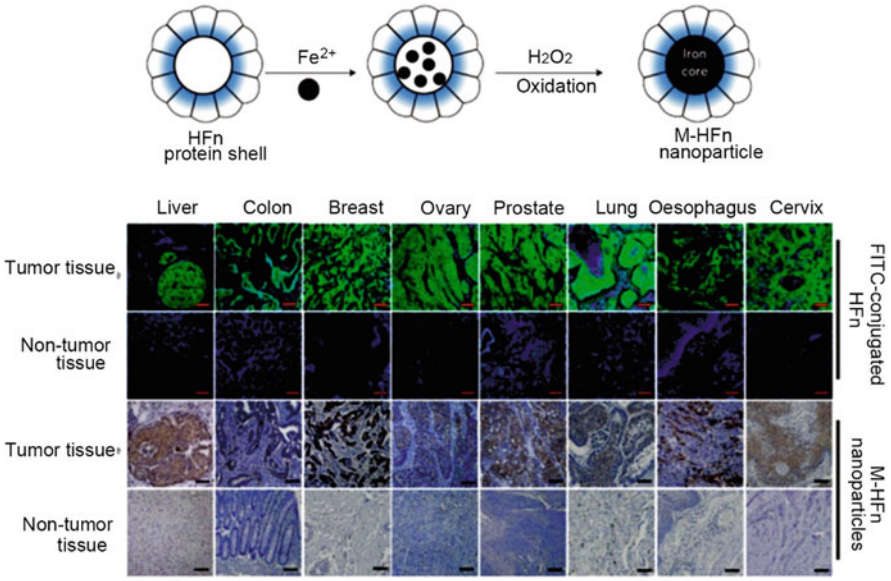

Fig. 9.8 Ferritin with peroxidase activity for tumor diagnosis [77]. (Reprinted from Ref. [77] with permission from the Nature Publishing Group)

9.3.3 In Vivo Oxidative Stress Regulation

Since Fe_3O_4 nanoparticles are often used for *in vivo* cancer imaging and therapy, their influence on cell viability needs to be thoroughly considered because there is intracellular H_2O_2 which can be catalyzed by Fe_3O_4 nanozymes. This may lead to changes in the levels of reactive oxygen species (ROS). Gu and coworkers found that Fe_3O_4 nanozymes perform dual enzyme-like activities, peroxidase and catalase, under acidic and neutral pH, respectively [16] (Fig. 9.9). Therefore the location of Fe_3O_4 nanozymes may lead to different outcomes in different intracellular microenvironments. Under conditions mimicking the lysosome, the nanozymes could catalyze H_2O_2 to produce hydroxyl radicals which induce glioma U251 cell damage. However, no hydroxyl radicals are produced under neutral conditions as found in the cytosol because the decomposition of H_2O_2 forms H_2O and O_2 directly under these conditions through catalase-like activity. These results provide a new way to evaluate the cytotoxicity of Fe_3O_4 nanozymes based on the intracellular location of nanoparticles. Besides the potential impact on ROS formation, Fe_3O_4 nanozymes may also cause liposome membrane damage due to lipid oxidation as reported by Wang et al. [20]. Fe_3O_4 nanozymes were found to catalyse pre-existing lipid peroxides (LOOH) or hydrogen peroxide as a substrate to initiate the chain reaction process at acidic pH via peroxidase activity. These results suggest another potential pathway to cellular oxidative damage, but this needs to be further investigated in cell models.

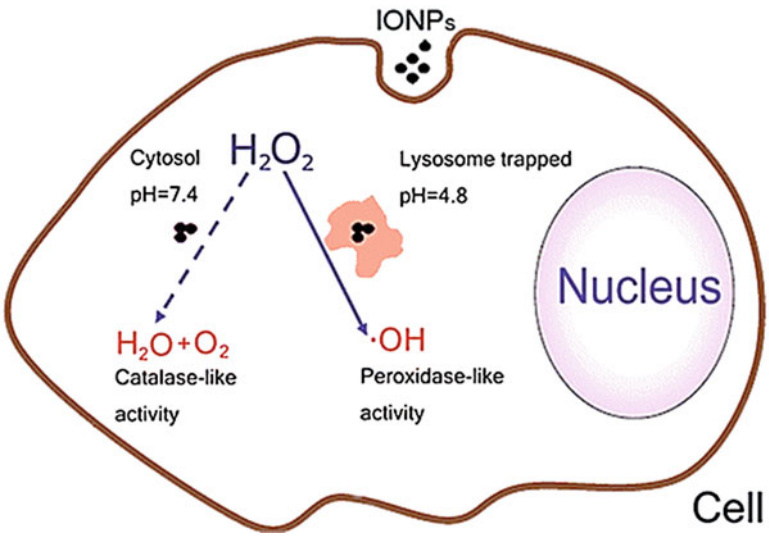

Fig. 9.9 Potential dual activities in the cell [16]. (Reprinted from Ref. [16] with permission from the American Chemical Society)

Despite potential cell damage, Fe_3O_4 nanozymes may provide cell protection by regulating ROS related oxidative stress. Huang et al. found that iron oxide nanoparticles could promote human mesenchymal stem cell (hMSC) proliferation [78]. They found that the commercial Ferucarbotran, an ionic superparamagnetic iron oxide (SPIO), could promote cell proliferation by diminishing intracellular H_2O_2. These nanozymes could also accelerate cell cycle progression. Similarly, Wang et al. found that poly(L-lysine)-modified Fe_3O_4 nanozymes could promote the proliferation of cancer stem cells from U251 glioblastoma multiform by reducing intracellular H_2O_2 [79]. Interestingly, Zhang et al. found that dietary Fe_3O_4 nanozymes could delay aging and ameliorate neurodegeneration in *Drosophila* through their intrinsic catalase-like activity [80] (Fig. 9.10). Fe_3O_4 nanozymes demonstrated the ability to protect cells from H_2O_2 induced oxidative stress and apoptosis. Furthermore, intracellular Fe_3O_4 nanozymes showed a neuroprotective effect in a Parkinson's disease (PD) cell model (PC12 cells originated from rat), which effectively inhibited α-synuclein accumulation and blocked caspase-3 activation. Fe_3O_4 nanozymes as a dietary supplement could enhance the climbing ability and prolong life span of aged *Drosophila* by reducing *in vivo* ROS. These nanozymes also alleviated neurodegeneration and increased longevity in an Alzheimer's disease (AD) model of *Drosophila*. All these investigations demonstrate that Fe_3O_4 nanozymes may perform beneficial functions including diminishing intracellular oxidative stress, delaying animal aging and protecting against neurodegeneration.

The cellular roles of Fe_3O_4 nanozymes relate to their biosafety when they are used for *in vivo* imaging or drug delivery. Although most studies indicate that Fe_3O_4 NPs have very low or negligible cytotoxicity, there is still concern about

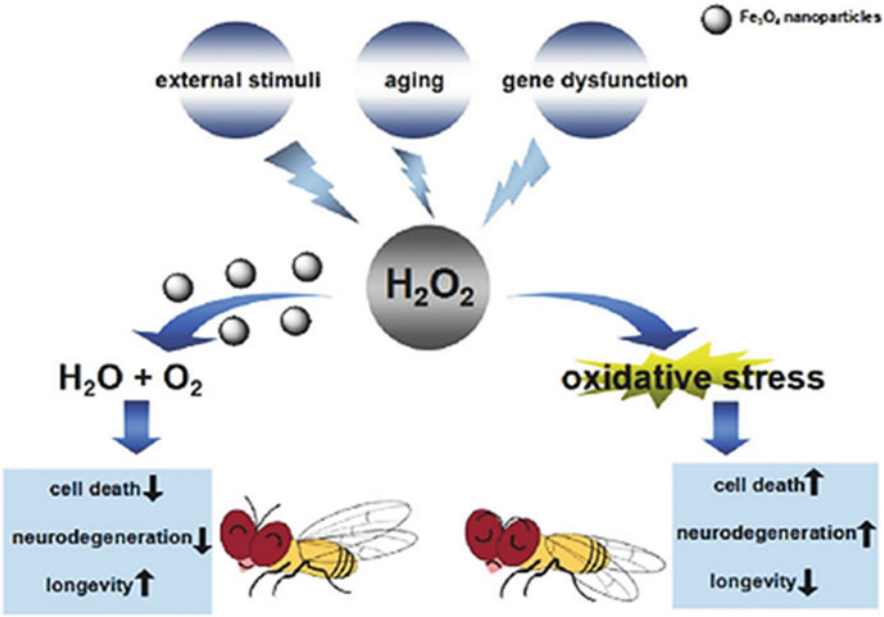

Fig. 9.10 Fe_3O_4 nanozymes delay aging and ameliorate neurodegeneration in *Drosophil*a [80]. (Reprinted from Ref. [80] with permission from and John Wiley & Sons Ltd.)

their biosafety, especially for long-term use. The pH-dependent enzyme activities of Fe_3O_4 NPs provide new understanding of their potential functions when taken up by the cell and it may be possible to control cell viability and fate via Fe_3O_4 nanozymes.

Besides acting as anti-ROS agents for neuronal protection, Fe_3O_4 nanozymes also show the potential for direct tumor destruction. Generally Fe_3O_4 nanoparticles are used for cancer imaging or targeted drug delivery. The enzyme-like activity of Fe_3O_4 nanoparticles is usually neglected in tumor therapy, but theoretically, this activity could affect tumor viability by catalyzing H_2O_2 to generate toxic radicals (Fig. 9.11). Zhang et al. have shown that magnetite Fe_3O_4 nanozymes can catalyze the decomposition of hydrogen peroxide to generate reactive oxygen species (ROS) to inhibit tumors *in vivo*, indicating their potential as a theranostic reagent for tumor therapy when combined with an enhanced T2-weighted signal in magnetic resonance imaging to target the tumor [81]. Here Fe_3O_4 nanozymes (13 nm in diameter) can be retained in the tumor microenvironment by an enhanced permeability and retention effect (EPR) and internalized by tumor cells via non-specific endocytosis. The combination of Fe_3O_4 nanozymes and H_2O_2 showed a significant inhibition effect on cell viability and more than 80% of HeLa cells died after treatment at different pH values. Furthermore, treatment with the combination of Fe_3O_4 NPs and H_2O_2 showed significant inhibition of tumor growth when applied to mice bearing subcutaneous HeLa tumors which often possess an acidic

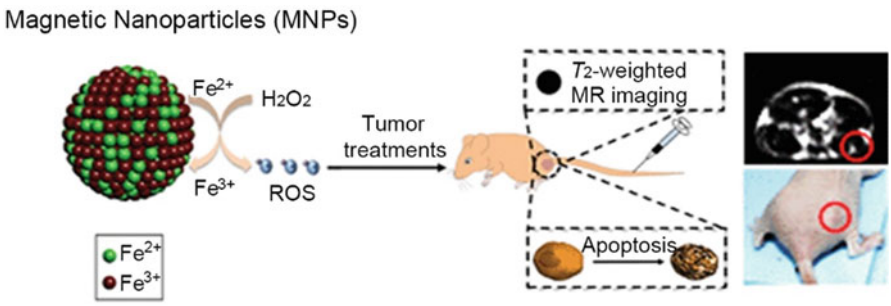

Fig. 9.11 Fe_3O_4 nanozymes for *in vivo* tumor diagnosis and therapy [81]. (Reprinted from Ref. [81] with permission from The Royal Society of Chemistry)

microenvironment which favors peroxidase-like catalysis, indicating that Fe_3O_4 nanozymes could be used for cancer theranostics, especially in epidermal diseases.

9.3.4 Hygiene and Dental Therapy

Hydrogen peroxide is a biocidal chemical that has various cleaning and disinfectant uses, including use as an anti-bacterial agent for hygiene and medical treatments. The mechanism is that H_2O_2 can release radicals slowly which damages cell membranes, proteins and nucleic acids. The addition of Fe_3O_4 nanozymes in the presence of its substrate H_2O_2 can boost the generation of hydroxyl radicals and thus enhance the antibacterial efficiency under acidic conditions.

Zhang et al. reported that Fe_3O_4 nanozymes combined with H_2O_2 have anti-bacterial activity towards *E. coli* [81]. A complete inhibition of *E. coli* proliferation (1×10^6 CFU mL^{-1}) was achieved in the presence of 20 μg mL^{-1} of iron oxide with diameter at 6 nm and 13.5 μg mL^{-1} of H_2O_2. In addition, Pan et al. designed a synergistic system by hybridizing reduced graphene with iron oxide nanoparticles (rGO-IONP). The rGO-IONP can effectively kill methicillin-resistant *Staphylococcus aureus* (MRSA) upon exposure to a near-infrared laser generating heat and hydroxyl radicals [82]. Animal experiments showed that the rGO-IONP promoted wound healing in the model infected with MRSA, indicating the system can be used as a general antibacterial strategy against drug- resistant bacteria.

The catalysis of H_2O_2 reduction to generate radicals also provides the opportunity to eliminate biofilms, which are generated by bacterial communities leading to drug resistance by limiting the penetration of antibiotics or other biocides into the protective, organic matrix of the biofilm (Fig. 9.12). Gao et al. found that Fe_3O_4 NPs with peroxidase-like activity could potentiate the efficacy of H_2O_2 in biofilm degradation and prevention via enhanced oxidative cleavage of biofilm components (model nucleic acids, proteins, and oligosaccharides) in the presence of H_2O_2 [84]. When challenged with live biofilm-producing bacteria, the Fe_3O_4 NP–H_2O_2

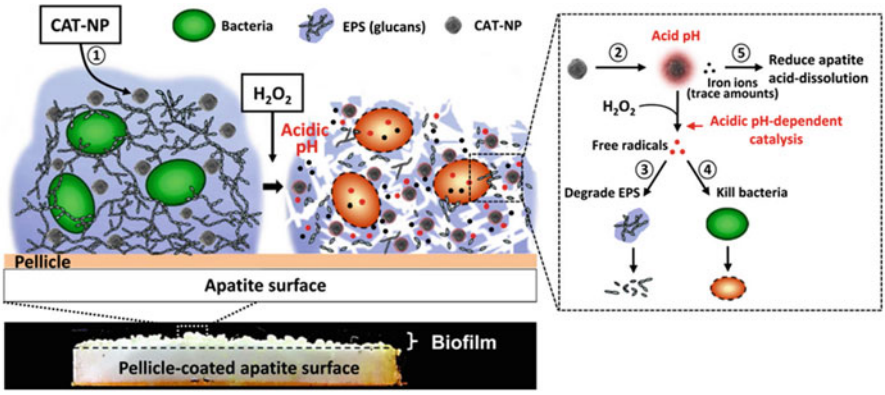

Fig. 9.12 Fe_3O_4 nanozymes for oral biofilm elimination and dental caries prevention [83]. (Reprinted from Ref. [83] with permission from Elsevier)

system efficiently broke down the existing biofilm and prevented new biofilms from forming, killing both planktonic bacteria and those within the biofilm, providing a novel strategy for biofilm elimination, and other applications utilizing oxidative breakdown. This strategy was successfully applied to dental biofilm elimination and caries prevention [83].

9.3.5 Eco Environment Applications

Besides biomedical applications, Fe_3O_4 nanozymes show great potential in environmental engineering, especially in hazard detection and removal. According to the catalytic reaction, the colorimetric signal is generated in proportion to the amount of H_2O_2 in the presence of chromogenic substrates. Therefore numerous applications have been focused on H_2O_2 detection since Wei and Wang first reported it [17, 59, 65, 72, 85–89]. H_2O_2 can be detected from many sources, including acidic rain [90], food, and living cells [91]. On the other hand, the signal is proportional to the amount of chromogenic substrates for a given amount of H_2O_2, which allows the detection of dyes in samples, such as certain pesticides [92], arsenic and antimony [93], 2,4-dinitrotoluene [94], beta-estradiol (beta-E-2) [95] and glutathione (GSH) [62]. Besides detection, catalysis of H_2O_2 decomposition by Fe_3O_4 nanozymes can also be used to degrade organic substrates for removal of pollutants, including phenol [23, 96–98], bisphenol [99, 100], aniline [22], methylene blue [101, 102], norfloxacin [103], xylenol orange [104], sulfathiazole [105] and Rhodamine B (RhB) [27, 106].

9.4 Summary and Future Perspectives

Fe_3O_4 nanoparticles show intrinsic enzyme-like activities including peroxidase- and catalase-like activities under acidic and neutral/basic pH, respectively. It should be noted that other types of iron oxide, such as Fe_2O_3, also possesses enzyme-like activities, but at lower levels than nanoscale Fe_3O_4 [9]. Therefore, Fe_3O_4 nanozymes are the main example of iron oxide nanozymes. Representing a new generation of mimetics, the catalytic properties and reaction kinetics of Fe_3O_4 nanozymes resemble natural enzymes. However, Fe_3O_4 nanozymes are much more robust and stable. More importantly, the activities of Fe_3O_4 nanozymes are tunable by controlling the size, morphology, nanostructure, dopants and surface modifications or by integration with other nanomaterials, which enables the rational design of nanozymes appropriate to the application of interest. Compared to traditional enzyme mimetics or natural enzymes, Fe_3O_4 nanozymes are multifunctional with intrinsic superparamagnetism and are readily functionalized with other molecules or labels. These features facilitate their use in a broad range of applications.

However, despite these many advantages, the activity of Fe_3O_4 nanozymes is still lower than natural enzymes and few studies on selectivity have so far been carried out. Therefore, the catalytic mechanisms need to be further investigated, especially in terms of nanoscale effects, in order to improve activity and selectivity by mimicking further features of the active site of natural enzymes. In addition, rational surface modifications are required to balance the activity and biocompatibility in biomedical applications. Finally, considering that more and more *in vivo* applications of Fe_3O_4 nanoparticles are being cdeveloped, their potential influence at the biochemical and cellular levels will be a new focus for nanozyme research (Fig. 9.13). We believe Fe_3O_4 nanozymes will have broad applications in biomedicine, industry and the environment, utilizing their intrinsic features and nanoscale effects synergistically.

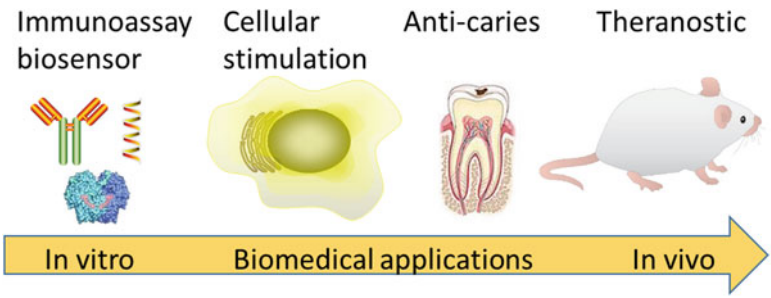

Fig. 9.13 The trend of Fe_3O_4 nanozyme applications from *in vitro* to *in vivo*

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