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Ferritin: a Powerful Platform for Nanozymes*

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Abstract Nanozymes have attracted increasing attention due to their potential applications related to targeted cancer therapy, diagnostic medicine, bio-sensing and even environmental toxicology. Ferritin with unique architecture, surface properties and high biocompatibility has emerged as an excellent and promising platform for the nanozymes. To highlight the significant progress of ferritin-based nanozymes research, this review discusses the functionality of ferritin in the field of nanozymes, including ferritins as templates/nanoreactors for the synthesis of nanozymes or nanozyme-catalyzed reactions, and as carriers for the delivery of nanozymes. We also try to address the current challenges ferritin-based nanozymes being confronted and point out future directions to reveal their latent potential.

Key words ferritin, nanozyme, template, nanoreactor, carrier

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Natural enzymes with a variety of catalytic activities are widely used in different fields due to its remarkable chemo-, regio-, and stereoselectivities, mild reaction conditions, and environmental compatibility^[1-3]. However, the practical application of natural bioorganic enzymes always limited by poor stability, strong environmental dependence, cumbersome purification process and recovery difficulties. Novel catalysts with high stability and low-cost are regarded as the alternatives to natural enzymes to overcome those limitations. Enzymes mimics, which are inspired by nature and aims to imitate the essential and general principles of natural enzymes using alternative materials, quickly become a hot spot for research and investigations. Although remarkable progress has been made in the development for decades^[4-9], utilization of most

enzyme mimics still faces several hampers. For example, the activity of most enzyme mimics is relatively low compared to that of their protein counterparts. More perishing is the poor substrate selectivity of enzyme mimics.

High-speed development of nanotechnology and emergence of numerous nanomaterials provide a new breakthrough in the development of enzyme mimics. Nanozymes, a term coined and accepted since the discovery of peroxidase-like activity of Fe_3O_4

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nanoparticles (NPs)^[10-11], are mostly adopt to describe nanomaterials with intrinsic enzymatic activities in recent years. From this perspective, nanozymes should be nanomaterials that can catalyze conversion of substrate under physiological conditions. The most important point is that enzymatic activities of nanozymes must come from the nanomaterial itself, rather than conjugating additional enzymes onto the nanomaterial^[12]. Consisting of mostly inorganic materials, nanozymes have several unique advantages over biological macromolecules, such as stability in both ambient and harsh conditions, more convenient mass production. Moreover, nanomaterials often exhibit very interesting magnetic, electrical, optical, and thermal properties, which is almost impossible for their natural counterparts to possess^[13]. These unique physicochemical properties and the well-known size-effects of nanomaterials endow nanozymes with diverse functionalities as well as more possibilities for their design. Therefore, nanozymes has gained a substantial development in the last ten years. Even though still in its infancy, nanozymes have already shown great application potentials in medical theranostics, bio-sensing, and even environmental toxicology^[14].

The catalytic properties of nanozymes depend mainly on the size, the surface morphology and chemical states, which plays critical roles in free radical generation, electron transfer, as well as substrate or products adsorption/desorption during the reactions. Therefore, it is necessary to prepare

nanozymes in a controlled manner for a narrow size distribution, defined surface and avertible agglomeration^[15]. Using biomolecules as pre-organized scaffolds has been demonstrated as an efficient approach to create chemically and spatially confined environment ideal for nanomaterials construction. Great efforts have been put to develop and apply a wide variety of nanomaterials utilizing diverse kinds of biomolecular templates^[16]. Viruses, virus-like particles, enzyme complexes, cellular micro-compartments, and other supramolecular proteins are superior for the construction of nanomaterials due to their high degree of symmetry, availability for modification and structural uniformity. In particular, ferritin has emerged as an excellent and promising protein-based nanocage to synthesize metal, metal oxide, semiconductor or precious metal nano-particles thanks to its unique architecture, surface properties and high biocompatibility^[17]. Although the progress and achievements of biomolecular-based nanomaterials have been thoroughly reviewed in the literature^[16, 18-19], no comprehensive review has been devoted to ferritin-based nanozymes. To highlight the significant research progress of ferritin-based nanozymes, this review discusses the functionality of ferritin in the field of nanozymes, including ferritin as templates/nanoreactors for the synthesis of nanozymes or the nanozymes catalyzed reactions, and as carriers for the delivery of nanozymes(Figure 1). We also will face the current challenges and try to give future directions to reveal the great potentials of ferritin-based nanozymes.

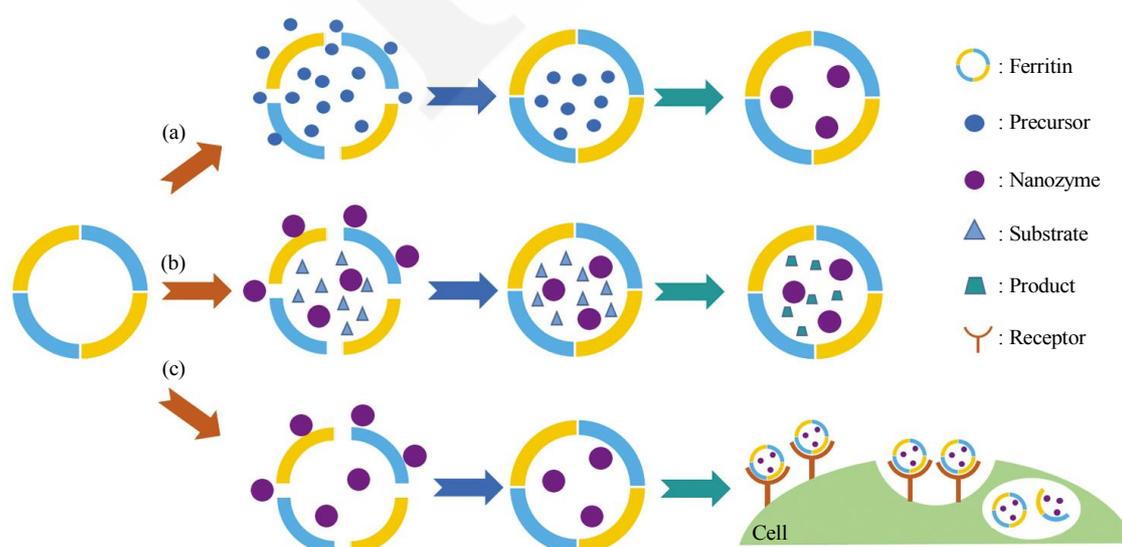


Fig. 1 The functionality of ferritin in the field of nanozymes

(a) Ferritins as templates/nanoreactors for the synthesis of nanozymes. (b) Ferritins as nanoreactors for the nanozyme-catalyzed reactions. (c) Ferritins as carriers for the delivery of nanozymes.

1 Ferritins

Ferritins are a superfamily of well-studied proteins that self-assemble into hollow cage-like structures and are ubiquitously found in animals, higher plants and various microorganisms (fungi and bacteria)^[20]. As a major iron storage protein ferritin can store up to 4 500 iron atoms in form of ferrihydrite within its protein shell^[21]. The ferritin protein without the iron mineral is called apoferritin. Proteins of the ferritin family can self-assemble into nano-cages of two types: maxi-ferritins and mini-ferritins. Maxi-ferritins are composed of twenty-four subunits, including both heavy (H-chains, 21 ku) and light (L-chains, 19 ku) chains. They self-assemble to form a spherical, cage-like structure that has inner and outer dimensions of ~ 8 and ~ 12 nm, respectively (Figure 2a). Each subunit is made up of a four-helix bundle with a short fifth helix at the C-terminus. The classical ferritins (Ftn) and the bacterioferritins (Bfr) are considered maxi-ferritins. The mini-ferritins (DNA-binding proteins from starved cells, Dps), of which hollow assemblies composed of twelve monomers, are tetrahedrally symmetric. The dodecameric mini-ferritins measures ~ 9 nm in diameter and has a central cavity of ~ 5 nm (Figure 2b). Similar to the maxi-ferritins, the mini-ferritins monomer folds into a four-helix bundle. However, unlike the maxi-ferritin, the loop between the B and C helices forms an additional short helix^[22-23].

The hollow cage-like structures endow ferritins a nanometer interior cavity as a separated microenvironment in which the interior reaction is independent of the external environment. The ferritin cage displays remarkable thermal and chemical stability and particularly amenable to reconstitution through controlled (dis)assembly^[15]. Such properties make ferritins one of the most popular supramolecular templates for the preparation of homogeneous nanoparticles with catalytic activities. The exterior surface of ferritins can be modified without altering the interior structures and characteristics, which make it possible for researchers to achieve controlled assembly of substances encapsulated by ferritins on a solid substrate to fabricate higher order structures^[23]. Furthermore, the biological properties and functions of ferritins enable the use of them as delivery agents for biomedical applications *in vitro* and *in vivo*. All these properties make ferritins attractive and powerful

platforms for the synthesis and application of nanozymes.

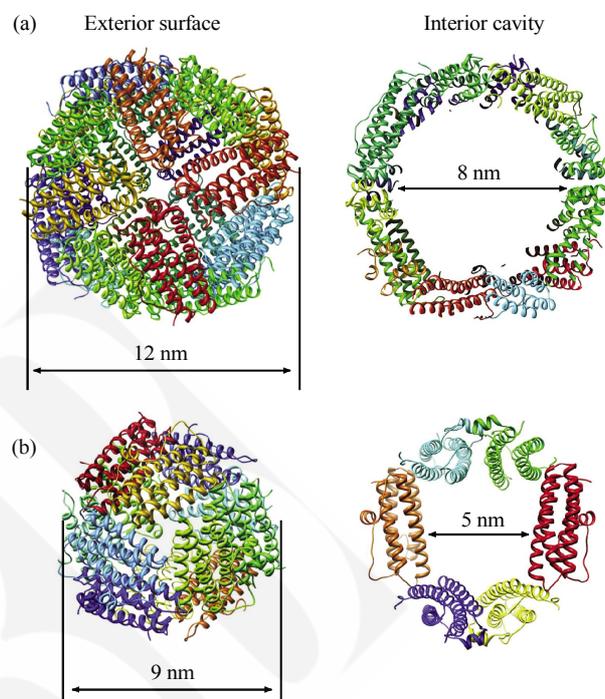


Fig. 2 Ribbon diagrams of exterior surface view and interior cavity

(a) Human heavy-chain ferritin. (b) *Listeria innocua* DNA-binding proteins. Reprinted with permission from ref. [23]. Copyright (2010) Elsevier.

2 Ferritins as templates/nanoreactors for nanozymes

Size control is an attractive feature of ferritin-templated synthesis which compared to other synthetic methodologies, produces nanoparticles with high monodispersities critical for catalytic activity. As mentioned above, the nanocage of ferritins provides a separated microenvironment in which the interior reaction is independent of the external environment, which make it an ideal reaction space for nanozyme-catalyzed reactions. In fact, it is easily found that the cavity of ferritin not only acts as template for the preparation of nanozymes, but also acts as nanoreactors for nanozyme-catalyzed reactions (Figure 1a, b).

Fe_3O_4 nanoparticles was the first inorganic nanoparticle was considered as an enzyme mimic with catalytic behavior similar to horseradish peroxidase^[10]. The loading and reduction of iron were successfully

carried out within recombinant human heavy-chain ferritin (HF_n) nanocages by Fan *et al.*^[24]. As expected, a well-defined iron oxide nanoparticles with an average diameter of ~ 4.7 nm was synthesized in HF_n which has well-defined morphology and is monodisperse in size. The synthesis procedure did not significantly perturb the overall protein cage architecture of HF_n. These magnetoferritin (M-HF_n) nanoparticles generated by encapsulating iron oxide nanoparticles inside a HF_n shell exhibited peroxidase activity towards typical peroxidase substrates such as 3, 3', 5, 5'-tetramethylbenzidine (TMB) and di-azo-aminobenzene (DAB) in the presence of H₂O₂.

Further study shown that the mineral phase composition of the iron core determines the peroxidase activity of the M-HF_n. While apoferritin, without a mineral core, exhibited no peroxidase activity, M-HF_n exhibited a much higher peroxidase activity. In comparison, natural holoferritin with cores mainly consist of the hydrated iron oxide mineral ferrihydrite (5Fe₂O₃·9H₂O), exhibits little peroxidase activity^[24].

Prussian blue nanoparticles (PBNPs) are usually considered as an “artificial enzyme peroxidase” because of their high surface activity and selectivity towards the reduction of hydrogen peroxide and oxygen^[25]. Zhang and coworkers^[26] report a simple method to synthesize PBNPs of very small size with horse spleen ferritin (HoSF) as the template, where the

PBNPs was synthesized by the reaction of ferrocyanide with the ferric iron at the surface of the ferrihydrite cores. The resulting Prussian blue modified ferritin nanoparticles (PB-Ft NPs) successfully combined the intrinsic enzyme mimetic activity of PBNPs and the specificity of ferritin with small size and relatively high catalytic activity. Furthermore, PB-Ft NPs displayed quite high sensitivity and affinity to H₂O₂ with ABTS as chromogenic substrate, which makes PB-Ft NPs a good reagent in glucose detection. PB-Ft NPs were successfully used in the ELISA, indicating that in PB-Ft NPs the antibody binding functions of ferritin were preserved, which make PB-Ft NPs a useful nanozyme label in biomedical detection.

Platinum nanoparticles (Pt NPs) resemble two biological enzymes, catalase and superoxide dismutase (SOD), which endow them the capabilities to quench hydrogen peroxide (H₂O₂) and superoxide (O²⁻). Zhang and coworkers^[27] showed that stable platinum nanoparticles can be successfully synthesized within the cavity of apoferritin and show catalytic reactivity for scavenging H₂O₂ and O²⁻ *in vitro*. The formation of Pt NPs with averaged diameter of approximately 2 nm was achieved by the chemical reduction of Pt(II) within the apoferritin cavity (Figure 3). And the SOD-activity increased almost 3-fold with the same protein concentration when Pt NPs were present inside the protein cavity (Pt-apo). It was also found that the

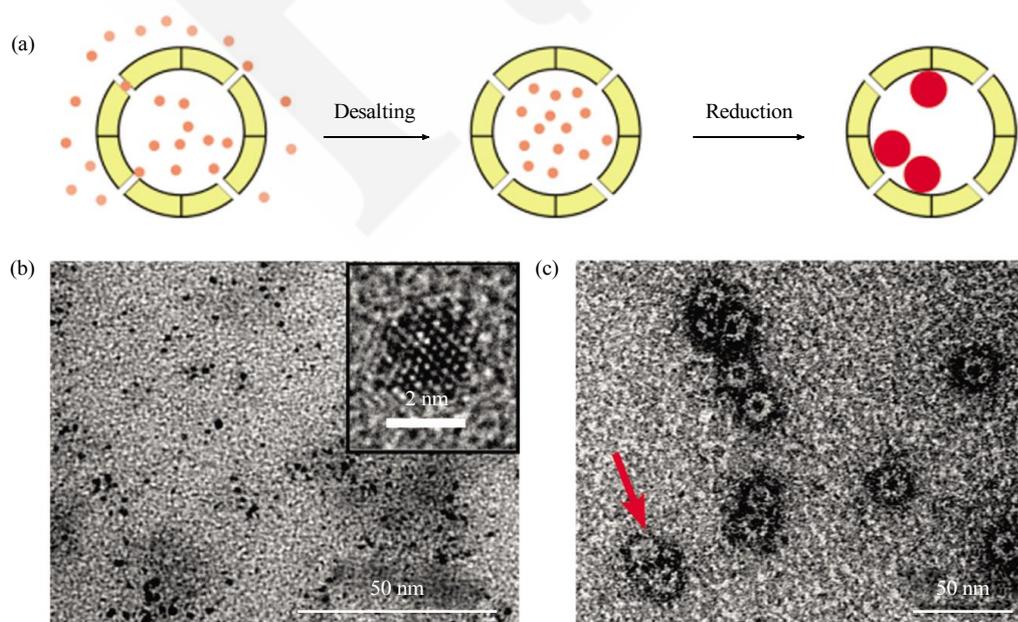


Fig. 3 Apoferritin-encapsulated platinum nanoparticles (Pt-apo)

(a) Preparation of apoferritin-encapsulated platinum nanoparticles. (b) TEM image of Pt-apo prepared from K₂PtCl₄/apoferritin with a molar ratio of 24 000:1. The concentration of K₂PtCl₄ in the mixture was 312 mmol/L (Inset: High resolution electron micrograph of a platinum nanoparticle). (c) TEM image of Pt-apo after negative staining with 1.5% uranyl acetate. The red arrow points toward an apoferritin without or with very small Pt-nps in the cavity. Reprinted with permission from ref. [27]. Copyright (2010) American Chemical Society.

enzyme mimic activities of Pt - apo could be regulated by inhibitor and reducing agent^[28]. For instance, the activities of Pt-apo can be inhibited by typical inhibitors of natural enzymes. 3-Amino-1, 2, 4-triazole (3AT) inhibited both catalase and SOD activities of Pt-apo, while NaN_3 inhibited only the catalase activity. These results demonstrated possibility of selective and specific suppression of nanozyme activities with inhibitors-another resemblance of nanozymes and enzymes. Interestingly, NaBH_4 could be used to recover inhibited catalytic activities of Pt-apo. This opens a door towards controlling the activities of nanozymes and provides a general technique. Using apo-ferritin as the scaffold, Nie and co-workers have also successfully synthesized highly stable Pt NPs (1–2 nm) with pH and temperature-dependent catalase and peroxidase activities^[29].

Gold clusters (Au clusters) below 2 nm in diameter have attracted great attention due to their unique intrinsic photoluminescence^[30] and selective catalytic activities^[31]. However, traditional routes for Au clusters synthesis are complicated and difficult to control, and the quantum yield is low. In order to overcome the above-mentioned difficulties, ferritins isolated from horse spleen were chosen as the nanoreactors for the synthesis of various paired Au clusters^[32]. To fine-tune the assembly of Au pairs within the ferritin shell, the authors were inspired by the well-known fact that elemental Au can be dissolved in aqua regia to generate Au^{3+} . They speculated that the reverse reaction could be used to form Au clusters from Au^{3+} by changing the reaction pH to basic. Especially, ascribe to the strong binding of Au^{3+} to the imidazole ring of the His residues, the His residues at the ferroxidase center of H-ferritin were utilized as “points of control” to grow Au cores in

this study. Though the resulting nanostructures were not used as nanozymes, this strategy provided a route for the position-controlled clusters of noble metal atoms of very small size (less than 1 nm). It is a very good reference for preparation of nanozymes utilizing ferritins as nanoreactors.

Metal alloy nanoparticles have unique sizedependent electronic, optical, and catalytic characteristics that are different from those of the single metal particles^[33]. Besides ferritins were utilized as template for the synthesis of single-metal nanoparticles, proteins in this superfamily are also known as the assemblies to accommodate various alloy nanoparticles^[34–36]. Therefore, researchers believe that the ferritins could be used to synthesize alloy nanoparticles for catalytic applications and it turned out that such ideas are really feasible.

Suzuki and coworkers^[37] reported the preparation of bimetallic Au/Pd core-shell and alloy nanoparticles in apo-rHLFr (recombinant L-chain apo-ferritin from horse liver) and the improved catalytic activity for olefin hydrogenations compared to Pd^0 nanoparticles in the apo-Fr cage (Figure 4). The AuPd (alloy) and [Au](Pd) (core/shell) NPs in the protein cavity show average particle sizes of (2.2 ± 0.2) and (2.4 ± 0.3) nm, respectively. This study also found that the catalytic activity of Pd located on the surface of [Au](Pd)-NPs can be improved by core Au nanoparticles in apo-rHLFr. PtAu alloy nanoparticles (PtAu-apo) also showed an enhanced activity towards hydrogen peroxide decomposition in comparison to the previously described apoferritin-encapsulated platinum nanoparticles (Pt-apo)^[38]. Similarly, the bimetallic Fe/Pt nanomaterials were synthesized in ferritin scaffolds and exhibit enhanced peroxidase activity compared to monometallic NPs^[39].

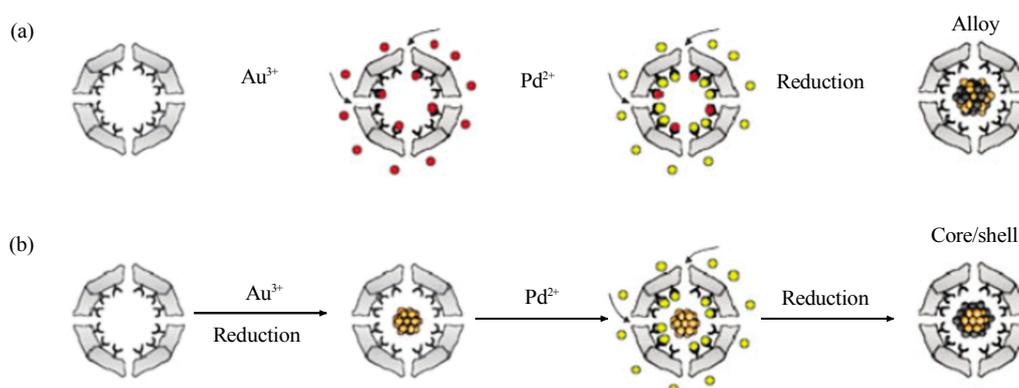


Fig. 4 Schematic drawings of methods of preparation of Au/Pd bimetallic NPs in apo-rHLFr

(a) Synthesis of (AuPd-NP)•apo-rHLFr and (b) synthesis of ([Au](Pd)-NP)•apo-rHLFr. Au^{3+} , Au^0 , Pd^{2+} and Pd^0 atoms are colored red, orange, yellow and brown, respectively. Reprinted with permission from ref.[37]. Copyright (2009) The Royal Society of Chemistry.

Homogeneous Au-Ag alloy nanoparticles have also been synthesized in the cavity of horse spleen apoferritin (HSAF) by a diffusion technique. The Au-Ag nanoparticle cores are 5.6 – 6.3 nm in diameter with narrow size distribution (≤ 1.0 nm), and their average diameter was gradually increased with an increase in the Ag content. The core formation ratios of Au-Ag-HSAF samples are higher than 80%. And the Au-Ag-HSAF samples showed strong catalytic activity on the reduction of 4-nitrophenol in the presence of NaBH_4 ^[40].

3 Ferritins as carriers for the delivery of nanozymes

Despite the extensive and intensive efforts at the preclinical level, however, few nanozyme-based drugs have been approved for clinical use. Major factors that impede the translation include toxicity and immunogenicity as many of the currently used nanocarriers are made of exogenously synthesized materials or contain heavy metals^[41]. Therefore, functional nanostructures with high biocompatibility and stability, low toxicity, and specificity of targeting desired organs or cells are of great interest in nanobiology and medicine. However, the biggest challenge is to integrate all of these desired features into a single nanobiostructure, which can be applied to biomedical applications and eventually in clinical settings. Physiologically, ferritins are ubiquitous cellular iron storage and detoxification proteins, and the surface of ferritins can be easily modified *via* either chemical or genetic approaches to introduce functionalities^[42-43]. Definitely, ferritins and its derivatives could be used as a powerful carrier for nanozyme-based drug (Figure 1c).

Iron oxide nanoparticles such as Fe_3O_4 nanoparticles can catalyze the oxidation of peroxidase substrates in the presence of hydrogen peroxide to produce a colour reaction similar to that of natural peroxidases^[10]. In addition, human transferrin receptor-1 (TfR1) was identified as an endocytosing cell-surface receptor of HF_n^[44]. TfR1 has a high affinity for HF_n and can mediate its specific binding to TfR1-positive cancer cells. In fact, HF_n has a universal capability for recognizing cancer cells. For example, HF_n exhibited significant binding to A375 melanoma cells, MDA-MB-231 breast cancer cells, K562 erythroleukemia cells, HeLa cervical cancer cells, SKOV-3 ovarian cancer cells, PC-3 prostate cancer

cells, U251 glioblastoma cells, U937 histiocytic lymphoma cells, SW1990 pancreatic cancer cells and Jurkat T-cell leukemia cells^[24]. Based on the properties of HF_n and iron oxide nanoparticles mentioned above, magnetoferritin (M-HF_n) was able to target, without any additional recognition ligands on their surface, and visualize tumor tissues through the peroxidase activity of the iron oxide core^[24]. M-HF_n nanoparticles strongly stained tumor cells and a clear distinction was seen between cancerous cells and adjacent normal cells in representative sections. This confirmed the clinical potential of M-HF_n nanoparticle-based diagnostic assay in cancer diagnosis.

Incomplete removal of excessive reactive oxygen species (ROS), which cause oxidative damage to human beings, leads to various detrimental effects on human health. Nanoceria particles (nano- CeO_2) drew much more attention due to their high ROS-scavenging activity and their reversibility and auto-regenerative properties^[45]. However, biocompatibility should be under consideration if the application of such nanozymes for disease treatment were desired. It is still a challenge to construct a highly active artificial enzyme with outstanding biocompatibility, although nano- CeO_2 more active than endogenous SOD. Liu and coworkers^[45] have demonstrated a strategy, combining ferritin with synthetic nano- CeO_2 , to construct a novel nano-complex (AFt- CeO_2). In their study, 4.5 nm nanoceria particles were successfully encapsulated into the apoferritin cavity *via* a dissociation-reconstruction route. The apoferritin encapsulation not only improves the biocompatibility and changes the cellular uptake route of nanoceria, but also manipulates the electron localization at the surface of the nanoparticle and thereby ameliorating the ROS-scavenging activity. To test whether cells adapted readily to AFt- CeO_2 treatment, the cellular internalization and cytotoxicity of AFt- CeO_2 were examined. It was found that although AFt- CeO_2 entered HepG2 cells efficiently, cells were still viable in the presence of a high concentration of AFt- CeO_2 . These results indicated a low cytotoxicity with a high internalized amount. This is a step-forward for the rational design/construction of nanozymes with designated functions, which have the potential to treat incurable diseases like some types of amyotrophic lateral sclerosis due to the defense failure against ROS.

Pt nanoparticles were reported to be useful ROS

scavengers which could protect the living organisms from any hazard caused by excessive ROS. Same as CeO₂, the bio-effects of Pt nanoparticles, especially potential toxicity, should be extensively addressed before their clinical application. Ferritins, again, become a priority option. Ferritin-platinum nanoparticles, which show good catalytic efficiency and long-term stability, were tested after ferritin-receptor-mediated incorporation in human intestinal Caco-2 cells^[27]. The H₂O₂-induced ROS in the cells decreased and the viability of the cells increased. Similarly, polyhedral Pt nanoparticles with uniform shape and narrow size distribution (average size of (4.3 ± 0.9) nm) were bio-mimetically prepared in the apoferritin cavity under mild synthetic conditions^[46]. Then, the internalization of AFt-Pt in HepG2 cells was observed. And the appearance of Pt nanoparticles in the cytoplasm, especially in the perinuclear regions of HepG2 cells confirmed the successful endocytosis of AFt-Pt. Moreover, the uptake kinetics profiles showed that AFt-Pt were preferentially ingested by HepG2 cells compared to PVP-Pt. The internalized amount of AFt-Pt was about three times that of PVP-Pt after incubation for 12 h^[46]. The researchers also proved that the apoferritin coating changed the internalization route and increased the cellular uptake amount of Pt nanoparticles *via* a harmless and native pathway, *i.e.* receptor mediated endocytosis. What's more, camouflage by apoferritin imparted a bio-recognizable identity to the particles, so that their biocompatibility was guaranteed. Apart from AFt-Pt, Liu and coworkers^[46] have also prepared apoferritin-encapsulated Au nanoparticles (AFt-Au), which also showed good biocompatibility and could be internalized into HpeG2 cells *via* receptor-mediated endocytosis.

Zhang and coworkers^[38] prepared PtAu nanoparticles with an average size of ~3 nm within apoferritin. The ferritin nanosphere served as a natural and biocompatible carrier for cellular delivery of bioactive materials through receptor-mediated endocytosis. Tests on Caco-2 cells showed that upon ferritin-based cellular uptake, PtAu-apo is a better mimic of cellular antioxidative enzymes than the monometallic Pt-apo. Cellular tests demonstrated that this biocompatible carrier enables the cellular delivery of catalytically active PtAu, which can be potentially applied in a biological environment, as a nanozyme to support cells to conquer oxidative stress^[38].

Impaired ferroxidase activity is one cause of the generation or progress of diseases. Under natural circumstances, the human body usually utilizes endogenous ferritin with ferroxidase activity as an antioxidant and for the iron depletion. However, its catalytic active sites are very sensitive to alterations in the microenvironment including, temperature, pH or ions. To avoid the disadvantage, Li and coworkers^[47] designed and constructed a stable and bioactive enzyme mimic by incorporating Pt nanoparticles as enzyme active sites into light-chain apoferritin. A stable ferroxidase with efficient iron mineralization ability was assembled from an inorganic ferroxidase and L-chain proteins. The nanoparticles oxidize ferrous ions to ferric ions, while the protein part mineralizes the ferric ions. In this system, light-chain apoferritin acts as carrier and collaborates with Pt nanoparticles. Such ferritin-based nanozymes with synergetic functions of ferritin and nanoparticles shows great promise for nanozyme design. All these results suggested that the apoferritin encapsulation is a universal approach to improve the bio-effects of nanozymes and shed light on the intracellular bio-effects of ferritin-encapsulated nanoparticles.

4 Assembly of ferritin

The basis for ferritins as templates, nanoreactors and carriers is the ability to self-assemble into a hollow structure. Therefore, an in-depth understanding of the disassembly and reassembly processes of ferritin is very beneficial to their application. A series of previous studies indicated that the reversible disassembly and reassembly of ferritins depend on the pH^[48-52].

To elucidate the structural information and the denaturation/denatured state of ferritins during the disassembly and reassembly processes, Kim and coworkers^[53] systematically investigated the 3D structures of horse spleen apoferritin under physiological conditions and their structural changes during disassembly and reassembly. It was found that apoferritin retains the highly ordered hollow sphere structure over a wide pH range 3.40 - 10.0. However, below pH 3.4, apoferritin becomes unstable with decreasing pH value and undergoes collapse and dissociation. For example, under pH 1.90 apoferritin underwent remarkable structural change from hollow sphere structure to disassembled oligomeric intermediates. Under extremely strong acidic condition

such as pH 0.80, the disassembled subunits further undergo aggregation (Figure 5). Besides, reassembly process of apoferritin by increasing pH was also examined. It was found that the pH-induced apoferritin disassembly and reassembly processes were not fully reversible. For instance, the results inform that the headset-shape structure of apoferritin (which was once formed at pH2.66 in disassembly process) is recovered

to a hollow spherical structure having two holes as defects but could not be recovered completely to the original intact hollow spherical structure under the neutral condition (Figure 5). The results of this study lend crucial insight into the assembly- disassembly mechanism of apoferritin and provide us important reference information for the utilization of ferritins in the field of nanozymes.

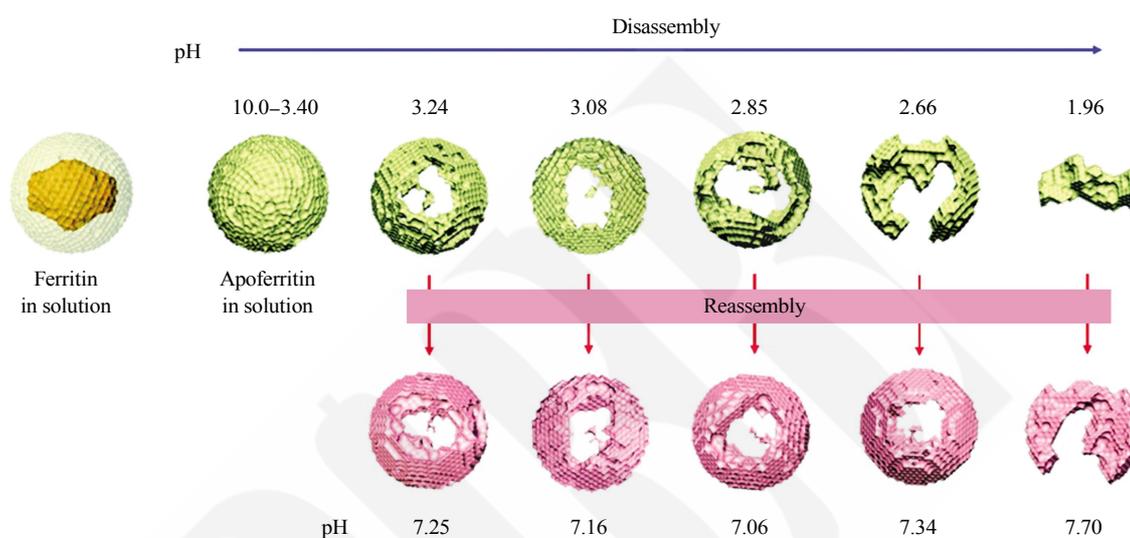


Fig. 5 pH-Dependent structures of ferritin and apoferritin in solution: disassembly and reassembly

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On the other hand, key interfacial residues at symmetry-related protein-protein interfaces that govern stability and self-assembly of a nano-cage maxi-ferritin (bacterioferritin from *Escherichia coli*) were determined through alanine-shaving mutagenesis^[54]. In this study, four amino acid residues (Arg-30, which is located at the two-fold axis, and Arg-61, Tyr-114, and Glu-128, which are located at the three-fold axis) were supposed to be oligomerization “switch residues”. Individual mutation of them to alanine completely shut down detectable solution formation of 24-mer, favoring a cooperatively folded dimer. Moreover, change of residue Arg-30 and Arg-61 to alanine form mutants that are more thermodynamically stable than the wild-type protein. This investigation into the structure and energetics of self-assembling nano-cage ferritin is a jumping off point for the eventual design of novel protein nanostructures.

Furthermore, due to extreme acidic conditions for ferritin disassociation is generally limited to the structures of bioactive compounds that are unstable at

such low pH, engineer ferritin molecules to make it disassemble at a higher pH value is of great importance to practical application. Chen and coworkers^[55] successfully fabricated a non-native ferritin-like protein (rHuHF- Δ DE), which can disassociate into subunits at pH 4.0 and reassembly at neutral. Such alteration was carried out by the cleavage of the last 23 amino acids (including DE turn and E helix) at the carboxyl terminal of ferritin which are involved in the 4-fold interactions. Interestingly, the above described cleavage almost has no effect on the assembly of ferritin shell-like structure, rHuHF- Δ DE can still self-assemble to form a shell-like protein cage.

5 Conclusion and perspective

Ascribe to their unique architecture, surface properties and high biocompatibility, ferritins have emerged as an excellent and promising platform for the nanozymes. In order to unfold the potential of ferritin in nanozyme technology, breakthroughs maybe achieved from further exploring its structural and

biological features.

Compared to natural protein enzymes, the inorganic nanozymes are still relatively poor in substrate selectivity due to the lack of specific bonding pockets for substrates. Therefore, the design and development of nanozymes with high selectivity towards given substrates is one of the greatest challenges. As protein-based templates/reactors for the synthesis of nanozymes, modification of interior surface of ferritins by small molecules or peptides may be an approach to enhance the selectivity of nanozymes. In addition, modification of the channels of ferritin cage can also be an option to control small molecules to pass through the protein shell. The channels could be designed by protein engineering as a preselection system, which allow the desired molecules enter and block the unwanted ones.

Inspired by the fact that natural enzymes work together as enzyme clusters in many cases. Coupling different nanozymes can be a way to fabricate multifunctional nanozyme systems for catalysis of chain-reactions. The functional assemblies of several nanozymes on the ferritin platform will lead to new paradigms with combined properties of different components, which implies the principal of proximity of natural multi-enzyme systems. In addition, the ferritin-based nanozyme system could combine with natural enzyme system to form a hybrid system which can integrate the function and advantages from both sides.

The biological functions of ferritins are far from being fully understood. Their roles in cancer, neurodegenerative and metabolism diseases remain unclear as before. Why ferritins are present in cell nuclei and mitochondria is still mysterious. The answers of these questions will definitely provide new possibilities for future applications of ferritin-based nanozymes. All in one, we believe that the continuing developments of ferritin-based nanozymes will lead to a new wave of novel catalysts for wide applications, especially in biomedical fields.

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铁蛋白: 纳米酶开发的重要工具*

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摘要 纳米酶因其在靶向癌症治疗、诊断医学、生物传感和环境毒理学等方面所具有的巨大应用潜力和价值而受到越来越多的关注。铁蛋白作为具有独特空间结构、表面性质和高生物相容性等特点的天然生物大分子, 已成为纳米酶开发的重要工具。为了展示和凸显铁蛋白在纳米酶开发中扮演的角色和取得的成就, 并为后续研究提供参考, 本综述着重介绍铁蛋白作为纳米酶合成的模板、纳米酶催化反应的反应器和纳米酶呈递的载体等。同时, 文中也指出了基于铁蛋白的纳米酶研发中所面临的挑战和其未来发展方向。

关键词 铁蛋白, 纳米酶, 模板, 纳米反应器, 载体

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