SCIENCE CHINA
Life Sciences

•INSIGHT•

November 2019 Vol.62 No.11: 1543–1546
https://doi.org/10.1007/s11427-019-1557-8

Nanozymes: an emerging field bridging nanotechnology and enzymology

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Received August 29, 2019; accepted October 8, 2019; published online November 5, 2019

Citation: Meng, X., Fan, K., and Yan, X. (2019). Nanozymes: an emerging field bridging nanotechnology and enzymology. Sci China Life Sci 62, 1543–1546. https://doi.org/10.1007/s11427-019-1557-8

Nanozyme, a class of nanomaterials with intrinsic enzyme-like properties, is a new concept which has been included in the Encyclopedia of China and the textbook of enzyme engineering. Since the first evidence published in 2007 (Gao et al., 2007), great progress has been achieved in the study of nanozyme from new concept, new material to its new application, and it becomes an emerging field bridging nanotechnology and biology (Gao and Yan, 2016). Nanozymes, like natural enzymes, can effectively catalyze the conversion of enzyme substrates under mild conditions and be used as natural enzymes for human health and environment (Huang et al., 2019a). However, unlike natural enzyme, nanozymes are generally low-cost, stable, and mass-produced (Wei and Wang, 2013; Wu et al., 2019). The study of nanozyme is not only to address the limitations of natural enzymes and conventional artificial enzymes, but also to inspire scientists to explore the intrinsic biological functions hidden in nanomaterials (Ragg et al., 2016), which may lead to a new field bridging organic and inorganic materials, and bridging nanotechnology and enzymology.

Nowadays, more than 540 nanozymes have been reported from 350 laboratories in 30 countries. Current studies of nanozymes mainly focus on the rational design of new types of nanozymes, catalytic mechanisms and their applications from potential to practical, from biomedicine to agriculture, forensic analyses, environmental protection, and even national security (Wu et al., 2019). This article will highlight the achievements in the study of nanozymes and its future trends (Figure 1).

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The rational design of new types of nanozymes

The 540 types of nanozymes reported so far can be divided into four categories based on the materials, i.e., metal-based, metallic oxide-based, carbon-based and other materials-based nanozymes. Based on the catalytic types, they can be divided into three categories: oxidoreductase family that includes peroxidase, catalase, superoxide dismutase and oxidase, hydrolase family that includes esterase, phosphatase, nuclease, protease, hydrogenase, and lyase family that includes carbonic anhydrase.

It is also found that the nanozyme activity could be tuned not only by its own physical characters such as size, morphology and composition but also by some environmental factors, for example, surface modification, ions, molecules and light. The physical characters of nanozymes determine the number of catalytic centers (Luo et al., 2010), nanocrystal facets (Fang et al., 2018) and electron transfer effi-

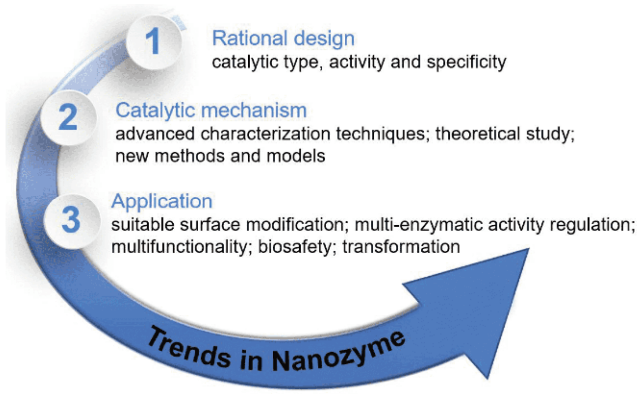

Figure 1 (Color online) Trends in nanozyme.

ciency (Mu et al., 2016), which are the basis of an enzymatic reaction. Environmental factors may tune the nanozyme activities by changing the binding affinity to substrate (Liu et al., 2017), blocking or forming new active sites (Long et al., 2011), stabilizing the product (Shah et al., 2015) or inducing pH and temperature change (Xu et al., 2014). These findings may guide the rational design of nanozymes, especially in solving the following two important issues.

Expanding the catalytic types of nanozymes

Natural enzymes are generally divided into six types: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. However, most of the current nanozymes are confined to oxidoreductases and hydrolases, and rarely found in the other four types. The discovery and rational design of new enzymatic types of nanozymes are not only important for their expansion, but also for their wider applications.

Improving the catalytic activity and specificity of nanozymes

Although nanozymes show advantages, the catalytic activities and substrate specificity of most nanozymes are still in need of improvement. It is encouraging that several approaches have been reported in improving nanozyme catalytic activities. By simulating the enzymatic microenvironment of natural peroxides via modification with a single amino acid on the surface of Fe_3O_4, Fan et al. (2016) improved the affinity of Fe_3O_4 nanozyme for its substrate thus improved its catalytic activity. By changing synthesis methods, Komkova et al. (2018) produced Prussian Blue nanozymes with 4 orders of magnitude higher peroxidase activity than natural peroxidase, through catalytic reaction involving H_2O_2 activation. Dong and co-workers mimicked the spatial structure of natural cytochrome P450 active centers using single-atom nanozymes with atomically dispersed enzyme-like active sites in nanomaterials, they achieved the highest oxidase-like activity among other nanozymes (Huang et al., 2019b). In addition, regulating the size, morphology and composition of nanozymes, or modifying functional groups on their surfaces might also help improve catalytic activities of nanozymes. As for the improvement of the specificity of nanozymes, we envision that the identification and catalysis of specific substrates can be achieved by modifying the suitable aptamers, chiral molecules or molecular imprinted polymers, which have targeted ability, on the surface of nanozymes, or combining nanozymes with natural enzymes that are selective to the substrate (Zhou et al., 2018).

Catalytic mechanisms of nanozymes

It has been found that the catalytic mechanisms of nanozymes are diverse. Although different types of nanomaterials exhibit similar catalytic activities, their catalytic mechanisms are usually different. In metal oxide nanozymes, their enzymatic activities of are usually from the conversion of metal ions between different valence states. For example, the peroxidase activity of Fe_3O_4 nanozyme involves the oxidation of substrates by intermediates $^{\cdot}OH$ (Zhang et al., 2008), while the reversible conversion of Ce^{3+} and Ce^{4+} in CeO_2 nanoparticle allows it to simulate superoxide dismutase and catalase (Celardo et al., 2011). In metal and carbon-based nanozymes, the enzymatic activities are closely related to the adsorption, activation and electron transfer of substrates. As for the peroxidase activity of graphene oxides, its carboxyl group is the binding point with the substrate ABTS, the carbonyl group is the catalytic position, and the hydroxyl group can inhibit the enzymatic activity (Sun et al., 2015). However, for the vast majority of nanozymes, the catalytic mechanism has yet to be revealed. Future work can be carried out in the following directions.

Advanced characterization techniques

In order to better understand the process and mechanism of nanozyme catalysis, it is important to develop advanced characterization techniques, such as in situ (sub) atomic resolution TEM imaging and synchrotron spectroscopies.

Theoretical studies

Theoretical studies play important roles in understanding nanozyme catalytic mechanisms. Using density functional theory calculation and experimental verification, scientists have analyzed the catalytic reaction process of nanozymes and the relationship between their structures and enzymatic activities, thus obtained the indicators to predict their activities (Shen et al., 2015). In future studies, this method can be used to establish a nanozyme database to provide theoretical support for the design, screening, optimization and application of nanozymes. Moreover, more theoretical methods should be introduced to mechanism studies because of the complexity of nanozymes.

New methods and models

Recently, some methods and models widely used in chemical catalysis were introduced to nanozyme research and provided new insight into nanozyme catalysis. For example, single nanoparticle collision electrochemical measurements have been used to study the catalytic activities of nanozymes at single nanoparticle scale (Hafez et al., 2019), this method not only unveils the intrinsic properties masked in ensemble-averaged measurements, but also explores the correlation between the structure and catalytic performance of nanozymes, which provides a deeper understanding of their catalytic mechanisms. The catalytic activity of single-atom nanozyme depends mainly on the well-defined structure of active centers, but not on factors such as size, morphology or composition, therefore single-atom nanozyme serves as an important model and provides a breakthrough in the investigation of nanozyme catalytic mechanisms. More methods and models are expected to be combined with nanozyme research to promote mechanistic studies in the future.

The applications of nanozymes

As a new generation of artificial enzyme, nanozymes have been widely used because of the following three characteristics. (i) Designability: the enzymatic activities of nanozymes can be adjusted by regulating their sizes, shapes, compositions and modifications to make them suitable for different practical applications. (ii) Multifunctionality: in addition to catalytic activities, nanozymes also have some unique physical and chemical properties in nanoscale, such as magnetism, fluorescence, electricity and photothermal effect, which make them multifunctional in biomedical applications. (iii) Applicability: nanozymes have the advantages of excellent stability, low cost and ease for mass production, which lay a valid foundation for their applications. Nowadays, the application of nanozymes has been extended to molecular detection and biological sensing, cancer diagnosis and treatment, oxidative reduction regulation of diseases, antibacteria and elimination of biofilms, environmental management and other aspects.

Despite the above promising properties, there are still challenges for nanozymes in their applications:

Balancing the surface modification and enzymatic activity of nanozymes

The catalytic activities of nanozymes are usually closely related to the atoms on the surface, but the functional groups of many nanozymes on their surfaces need to be modified in order to increase stability, improve dispersion or obtain targeting ability. These surface modifications can alter the surface properties of nanozymes, which may affect their enzymatic activities. Therefore, how to balance the surface modifications and enzymatic activities of nanozymes is a key concern in their application.

Regulation of multi-enzymatic activities of nanozymes

Nanozymes usually exhibit multi-enzymatic activities, thus, how to selectively stimulate them to exhibit desirable enzymatic activities becomes an important issue, as deviating from the activities of the design may lead to an opposite effect. Recently, our group modified ferritin on the surface of nanozymes, and the modified nanozymes can be selectively guided into acidic lysosomes of tumor cells and exhibit their peroxidase and oxidase activities (Fan et al., 2018). In this way, tumor catalytic therapy can be achieved. This work provides a new approach to selectively activating the specific enzymatic activities of nanozymes. More strategies need to be developed for different nanozymes and different needs.

Development of the multifunctionality of nanozymes

It is important to develop multifunctional nanozymes by combining the catalytic activities of nanozymes with their physical and chemical properties. The multifunctional nanozymes will expand the applications of nanozymes and create more advanced products.

Safety concerns for in vivo application

Newly developed nanozymes as therapeutics like other nanomaterials, must meet strict biosafety and efficacy requirements. Nanomedicine (nanodrug delivery system) is widely studied (Zhang et al., 2018a, b; Hameed et al., 2018; Ran and Xue, 2018), and FDA has approved an increasing number of nanomaterials for imaging and therapeutic applications, including the widely studied iron oxide nanoparticles and Prussian blue nanoparticles. Iron oxide nanoparticles act as an iron supplements and magnetic resonance imaging contrast agents, while Prussian blue nanoparticles serve as the antidote of thallium elements. Although most nanozymes used *in vivo* research lack systematic biosafety evaluations, there is great hope of developing nanozymes as diagnosis and therapeutic drugs due to the similarities between nanozymes and FDA-approved drugs.

Obviously a systematic research on the safety of nanozymes after they are taken into the body is needed, including their distribution, absorption, pharmacokinetics, metabolism, duration of therapeutic effect and excretion.

Transformation of research achievements

Due to the excellent performance of nanozymes in their applications, more efforts should be put into the development of nanozyme products and their industrial research. The nanozyme-strip, a medical device for the detection of virus or other biomolecules, is a model for successful nanozyme transformation and has been approved by China Food and Drug Administration in 2018. We expect that nanozymes will not only be widely used in basic research, but also truly improve human health and quality of life.

In conclusion, despite the remarkable progress that has been made in nanozyme research, there are still many challenges to be addressed in the future. Close collaboration of scientists from multidisciplinary areas will be needed to develop new types and more effective nanozymes, establish the theory of nanozyme catalysis and promote their application research.

Compliance and ethics *Compliance and ethics The author(s) declare that they have no conflict of interest.*

Acknowledgements *This work was financially supported by the Strategic Priority Research Program (XDB29040101), the National Natural Science Foundation of China (31900981, 31871005, 31530026), Chinese Academy of Sciences (YJKYYQ20180048), the Key Research Program of Frontier Sciences (QYZDY-SSW-SMC013), Chinese Academy of Sciences and National Key Research and Development Program of China (2017YFA0205200), and Youth Innovation Promotion Association CAS (2019093).*

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