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Nanozymes: an emerging field bridging nanotechnology and biology

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Enzymes are biological catalysts that can convert substrates into products in biochemical reactions. In 1926, the first enzyme, urease, was determined to be a protein by James B. Sumner who won the Nobel Prize in 1946. Since then, enzymes have been considered to be proteins, which allows them to achieve their high catalytic activity with high specific activity under mild conditions. However, in general, the enzyme activity of proteins is lost after exposure to extremes of pH and high temperature, and proteins are also susceptible to digestion by proteases in the environment, which dramatically hinders their practical applications in industry. To overcome the limitations, there is increasing interest in enzyme mimetics, which are more robust than proteins and easier or more economical to produce. The majority of enzyme mimetics use organic chemical molecules as the designed backbone, following the host-guest concepts of Donald J. Cram, Jean-Marie Lehn, and Charles J. Pedersen who won the Nobel Prize for chemistry in 1987. In addition, non-protein biomolecules with enzyme-like activities have been discovered, such as Ribozymes (1989 Noble Prize awarded to Thomas R. Cech and Sidney Altman), Abzymes and DNAzymes. Although under development for decades, most enzyme mimetics are still limited in their practical applications because of their low activity and selectivity. Therefore, discovery of new enzyme mimetics is necessary.

Nanozymes, a new type of enzyme mimetic, are nanomaterials with intrinsic enzyme-like activity, which can efficiently catalyze conversion of substrate and follow the same kinetics and mechanism of natural enzymes under physiological conditions. Here we specify that the enzyme-like activities of nanozymes must come from the nanomaterial itself, rather than conjugating additional enzymes onto the nanomaterial. In 2007, our group provided the first evidence that inert ferromagnetic nanoparticles have intrinsic peroxidase-like activity (Gao et al., 2007), which quickly aroused attention in nano-research and biology. So far there are more than 50 kinds of nanomaterials that have been found to possess intrinsic activity similar to enzymes such as peroxidases, oxidases, glucose oxidases, haloperoxidases, superoxide dismutases and sulfite oxidases (Gao and Yan, 2013; Ragg et al., 2015). This discovery has changed the idea that nanomaterials are chemically inert in biological systems, and directly led to the new concept that nanomaterials are bioactive. Soon after that, scientists started to use these new characteristics of nanoparticles to make biosensors for glucose detection, and in tumor diagnosis and environmental treatment (Gao and Yan, 2013). In 2013, Erkang Wang and Hui Wei reviewed nanomaterial-based artificial enzymes and indicated that nanozymes represent the next generation of artificial enzymes (Wei and Wang, 2013). Since then, the nanozyme paradigm, referring to nanomaterials with intrinsic enzymatic activity, has led to

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a newly emerging field bridging nanotechnology and biology (Shin et al., 2015).

As enzyme mimetics, nanozymes possess intrinsic enzyme-like activity, which can catalyze the conversion of substrates to products, showing similar catalytic kinetics and mechanism as natural enzymes. For example, the Fe_3O_4 nanoparticles, like peroxidases, catalyse the oxidation of different peroxidase substrates such as 3,3′,5,5′-Tetramethylbenzidine (TMB), 3,3′-Diaminobenzidine (DAB), and o-Phenylenediamine (OPD) to give the same color changes. The peroxidase-like activity of Fe_3O_4 nanoparticles is also dependent on pH, temperature and H_2O_2 concentration. Interestingly, the catalysis by Fe_3O_4 nanoparticles, like peroxidase, shows typical Michaelis-Menten kinetics, and is consistent with a Ping-Pong mechanism. However, nanozymes are more stable than enzymes because they are inorganic nanomaterials. In addition, as the properties of nanoscale materials are often dependent on size, the catalytic activity of nanozymes is tunable by controlling size, structure, dopant, morphology and surface modification. This is an advantage that previous enzyme mimetics lacked. Importantly, nanozymes have the additional property of being nanomaterials. For example, Fe_3O_4 nanoparticles have two intrinsic properties, namely magnetism and peroxidase activity, which makes them a dual functional molecule, magnetism for separation and catalysis for signaling (Figure 1). Therefore, the versatility and power of nanozymes opens up a wide range of new potential applications in environmental chemistry, biotechnology and medicine.

Nanozymes not only provide new examples of enzyme mimetics, taking this field from supramolecular chemistry to nanomaterials, but also allow for new practical applications of enzyme mimetics due to their highly effective catalysis and versatile functions. For example, a nanozyme-strip could detect Ebola virus with 100 times higher sensitivity than standard strips, using the superparamagnetism and peroxidase activity of a nanozyme to concentrate antigen and amplify the signal simultaneously (Duan et al., 2015). Similarly, a magnetoferritin nanozyme shows great potential for rapid cancer diagnosis by combining tumor specific recognition (ferritin) and coloration (peroxidase) into a one-step reaction (Fan et al., 2012). In addition, conjugating glucose oxidase (GOx) onto iron oxide nanoparticles can detect glucose levels quickly and easily via sequential reactions from GOx to the nanozyme (Wei and Wang, 2008). This is the first time that nanomaterials have been used as an alternative to enzymes for in vitro immunoassays and biosensors. Meanwhile, there is increasing attention on probing the in vivo effects of nanozymes in biological systems from bacteria and mammalian cells to animals. The V_2O_5 nanozyme (haloperoxidase) shows antibacterial activity preventing biofilm growth in marine antifouling (Natalio et al., 2012) and carbon nanozmyes (peroxidase), like carbon nanotubes, graphene oxide and carbon dots, also show potential antibacterial effects in wound recovery (Sun et al., 2014). In comparison, iron oxide nanozymes with dual activities (peroxidase and catalase) may affect cell viability via the enzymatic activities taking place intracellularly (Chen et al., 2012). More interestingly, these iron oxide nanozymes could effectively delay aging and neurodegeneration in *Drosophila* (Zhang et al., 2016) and significantly reduce oral biofilm and synergistically prevent dental caries in a rat model (unpublished).

As a young field, there are still a lot of unknowns and challenges regarding nanozymes. First, it will be interesting to answer why different kinds of nanomaterials, such as Fe_3O_4, gold and silver nanoparticles, carbon nanotubes and graphene oxide can all show the same activity, such as peroxidase activity. Also, why one nanozyme shows two or more different kinds of enzyme-like activities. For example, F_3O_4 nanoparticles at low pH show a peroxidase-like mechanism but a catalase-like mechanism at neutral pH (Chen et al., 2012). Better understanding of the fundamental mechanisms behind all these phenomena will help guide the design of further nanozymes. Secondly, how to improve the selectivity and/or specificity of nanozymes is another important issue. Chemical modification of the surface of nanozymes was found to improve the substrate affinity (K_m) but did not improve the selectivity. The solution may require reference to the structural information in naturally

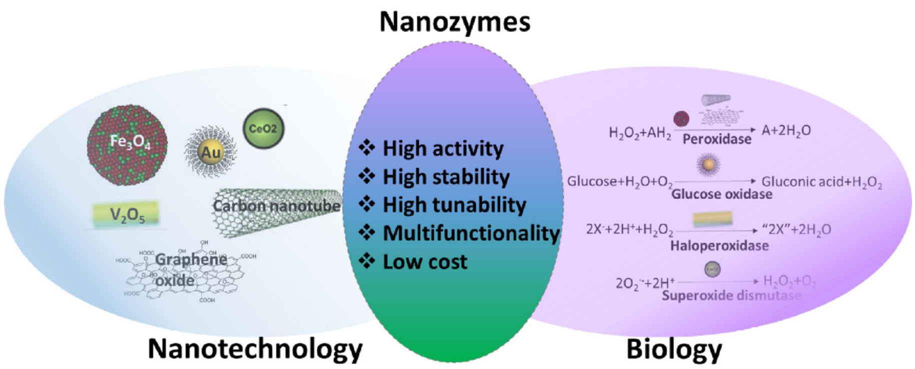

Figure 1 Nanozymes as an emerging field bridging nanotechnology and biology.

occurring enzymes in order to find a successful strategy to introduce it into nanozymes. Thirdly, further types of nanozymes should be explored. Up to now, although 50 kinds of nanomaterials have been found with intrinsic activities, most have oxidoreductase-like activities such as peroxidase and oxidase. However, in nature, enzymes catalyze more than 5,000 biochemical reactions, which are generally classified into six types: oxidoreductases, transferases, hydrolases, lyases, isomerases and ligases. Therefore, there is great potential for exploring nanozymes with a wider range of activities. To achieve this, we require more thorough understanding of the fundamental mechanisms of nanozymes, and to combine this creatively with structural information for natural enzymes. Thus, exploring nanozymes with a wider range of enzymatic activities is greatly anticipated. Fourthly, to widen the application of nanozymes, it is important to synergistically combine their enzymatic activity with other special nanoscale properties such as magnetics, optics, electrics and mechanics. The multifunctionality of nanozymes will facilitate the creation of state-of-the-art technologies and products which can be used to improve human health and quality of life. Overall, we believe nanozymes have already become a highly active interdisciplinary field bridging nanotechnology and biology.

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

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