

Functional Nanozyme

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Contributor: Nan Cheng

Nanozymes have the potential to replace natural enzymes, so they are widely used in energy conversion technologies such as biosensors and signal transduction (converting biological signals of a target into optical, electrical, or metabolic signals). The participation of nucleic acids leads nanozymes to produce richer interface effects and gives energy conversion events more attractive characteristics, creating what are called “functional nanozymes”.

Keywords: nanozyme ; nucleic acid ; modulation ; recognition ; energy conversion

1. From “Enzyme” to “Nucleic Acid Modulation for Functional Nanozyme”

1.1. Enzyme

In the field of biology, enzymes are a class of macromolecular substances with biocatalytic functions. Only under suitable temperature and acid-base conditions can enzymes change the physiological state of organisms by regulating their metabolic pathways, such as by signal transduction, gene expression, gene silencing, etc., or by obtaining new biological traits or removing certain biological traits^{[1][2]}. Enzymes usually exhibit excellent catalytic efficiency and regio- and stereoselectivity, and they are widely used in biochemical energy conversion, playing an important role in food risk-factor detection, medical disease diagnosis and treatment, and environmental pollutant analysis^{[3][4]}.

Most natural enzymes are proteins or RNA, and some studies have pointed out that enzymes can also be DNA. Natural enzymes lose their activity when encountering nonphysiological conditions, and the preparation process of enzymes is complicated and expensive^{[5][6]}. Therefore, in the past few decades, scientists have been seeking to synthesize compounds with properties similar to enzymes' activity by chemical or physical methods. These compounds are stable, economical, and able to adapt to nonphysiological conditions to solve problems in practical applications^[7].

1.2. Nanozyme

In recent years, nanoparticles (NPs) with strong tolerance to the external environment have shown dual characteristics, including biological properties and material chemical properties, and these NPs are called “nanozymes”, which are expected to replace natural enzymes^{[8][9]}. In 2004, self-assembly triazacyclonane-functionalized thiols on the surface of gold NPs exhibited RNase-like behavior that catalyzed the cleavage of phosphate esters^[10]. Then, to define this layer of gold nanoclusters, scientists proposed the concept of a “nanozyme”. NPs with enzymatic activity were later described as nanozymes^{[11][12]}. In 2007, Yan et al. reported for the first time that Fe₃O₄ nanoparticle (NP) catalysts showed properties similar to peroxidase^[13]. The publication of this work changed the traditional concept of inorganic nanomaterials being biologically inert substances, revealed the inherent biological effects and new characteristics of nanomaterials, and expanded from organic composites to inorganic nanomaterials, which broke the boundary between “inorganic” and “organic” in the traditional sense.

Moreover, by combining excellent physical and chemical properties with enzyme-like catalytic activity, nanozymes can realize multifunctional biological applications from detection to monitoring and treatment and have been widely studied in the fields of medicine, chemistry, food, agriculture, and environment^[14]. Compared with natural enzymes, nanozymes have the following characteristics^[14]:

(1) High stability: Inorganic nanomaterials are more adaptable to pH and temperature changes than natural enzymes. Some nanozyme can be used under a wide range of pH (3–12) and temperature (4–90 °C) conditions. In contrast, biological enzymes are usually easily denatured and inactivated under extreme pH and temperature conditions.

(2) Low cost: The production process of enzymes is usually complicated and expensive, while inorganic nanomaterials are easy to produce on a large scale with good catalytic activity and low cost.

(3) Recycling: Inorganic nanomaterials are recyclable, and there is no significant loss of catalytic activity in the subsequent cycles.

(4) Easy to be multifunctional: nanozymes have a large specific surface area and high surface energy and can be combined with multiple ligands to achieve multifunctionality^{[15][16][17][18]}.

Nanozymes, as new stars in science, not only have the characteristics of materials chemistry, including a large specific surface area, rich surface morphology, easy modification, and unique size and shape, but also have more attractive biological characteristics, including the ability to respond to physiological reactions and to catalyze biochemical reactions^{[19][20][21][22][23][24][25]}.

1.3. Functional Nanozyme

Due to the excellent dual properties of nanozymes, some specific studies have shown that they can respond well to the changes of biological macromolecules (proteins, nucleic acids, polysaccharides, lipids).

(1) As for proteins, Zhang et al.^[26] encapsulated transition metal catalysts (TMCs) on the single-layer surface of gold nanoparticles to prepare a bio-orthogonal nanozyme. By weakening the formation of a constant protein crown (hard corona), the long-term retention of nanozyme activity in the cell is achieved.

(2) As for nucleic acids, Wang et al.^[27] found that ssDNA adsorbed on g-C₃N₄ NSs could improve the catalytic activity of the nanosheets.

(3) As for polysaccharides, Li et al.^[28] synthesized soluble molecularly imprinted nanozyme that can accurately hydrolyze the oligosaccharide maltohexaose.

(4) As for lipids, Zhang et al.^[29] reported magnetic nanoparticles (iron oxide nanozyme). After a nanozyme enters the cell, it exerts peroxidase activity in the acidic environment of the lysosome, increases the level of ROS activity, destroys proteins, nucleic acids, lipids, and other biological molecules, makes them lose their functions, and kills *Escherichia coli*.

Overall, the structure and surface physicochemical properties of a nanozyme determines whether it has the characteristics to cope well with biological macromolecules' changes and thus to produce unique nanobiological effects.

Among these biological macromolecules, nucleic acids have been known for specific self-assembly properties and unique molecular recognition mechanisms^[30]. First, the double-stranded structure of nucleic acids has complementarity, and a series of DNA-based nanomaterials can be developed based on this complementarity between strands^[31]. Moreover, as a biological recognition molecule, a nucleic acid aptamer is essentially a single-stranded DNA or RNA folded to form a specific secondary and tertiary conformation that then binds to the target molecule with high affinity and specificity^[30].

The increasing development of nucleic acid technology has promoted the progress of studies on biochemical energy conversion related to biomacromolecule-modulating nanozymes (energy conversion: the energy generated by the biochemical event is transformed into other forms of energy, such as light energy, electric energy, new biological energy, or new chemical energy)^{[32][33][34][35][36]}. Specifically, nucleobases can provide lone pairs of nitrogen and oxygen electrons, and nucleobases are an important structure of the nucleic acid phosphate backbone. Thus, nucleic acid acts as a multidentate organic ligand, which interfaces with metal ions, metal oxides, metal organic frameworks, and carbon bases of nanozymes, transferring electrons to form functional nanozymes^{[37][38][39][40]}. The interaction between functional nanozymes and various interface components was analyzed by generating energy conversion effects (biosensing and signal transduction)^[41]. Like target recognition conversion into an optical signal or electrical signal, the probe assembled by gold nanozyme and aptamer AG3 converts the process of specific recognition of murine norovirus into an optical signal^[42]; the probe assembled by the gold nanoparticle–graphene oxide hybrid and the respiratory syncytial virus antibody converts the process of specifically recognizing RSV into an optical signal^[43]. Like target substance activation conversion into a metabolic signal, Fe₃O₄ NPs induce AMPK activation and enhance glucose uptake, which has potential effects in diabetes care^[44]; organic polymer nanozyme SPNK induces the release of KYNase, which degrades kynurenine (Kyn)^[4]. Consequently, it may be true that nanozymes can control immunomodulation. By studying the interface effects of nanozymes in these energy conversion events and analyzing the interactions between various interface components, we further understand functional nanozymes^[41].

1.4. Nucleic Acid Modulation for Functional Nanozyme

Since one of the centerpieces of the biochemical energy conversion mechanism is the interface modulation event, more attention has focused on the strategy of interface modulation for target assay. To the best of our knowledge, this article is the first to analyze and summarize such phenomena. This article pays great attention to the interface modulation of nucleic acids to nanozymes: modification, binding, immobilization, and the resulting major changes in interface components in the structure of nanozymes, which lead to an increase or decrease in enzyme catalytic activity^{[45][46][47][48][49]}. The controllability and accuracy of modulation technology is an important force for promoting the progress of social civilization. To cater to the perfect control of enzyme activity in practical applications, scientists have expended great effort in studying the crosstalk between nanozymes and nucleic acids, which retains the advantages of nucleic acids but does not limit the properties of nanozymes that need to be expressed.

2. Crosstalk between Nanozyme and Nucleic Acid

Nucleic acid modulation of nanozymes is a key step in energy conversion events. Studies have shown that nucleic acids can change the size, shape, composition, surface modification, and state of NPs through biological, chemical, and physical effects^{[50][51][52][53]}. Here, we scientifically discuss the construction of three types of crosstalk between nanozymes and nucleic acids: (nanozyme precursor ion)/(nucleic acid) self-assembly, nanozyme-nucleic acid irreversible binding, and NP-nucleic acid reversible binding.

2.1. Self-Assembly Nanozyme

The rich structural features of nucleic acids endow them with diverse binding capabilities with NPs. Due to the noncovalent interactions that occur during the assembly of NPs and nucleic acids, the assembled nanozymes have new interface effects and exhibit the desired morphology, chemical-physical properties, and stimulus responsiveness.

Here, we summarize the interactions among components. Recent studies have confirmed that the presence of various elements commonly found in nuclein—oxygen and nitrogen^[54]. Nitrogen acts as an electron donor, and oxygen provides electron pairs, which can hybridize or covalently coordinate with single or multiple metal ion precursors (Figure 1); therefore, they can controllably modulate the enzyme activity and stability of nanoparticles^{[55][56][57][58][59]}. In addition, Chen et al.^[58] introduced nanocarrier mesoporous silica based on the study of noncovalent binding and discovered that the nucleic acid acted on the platinum particle precursor ion to produce a “reversible” masking effect at the active site.

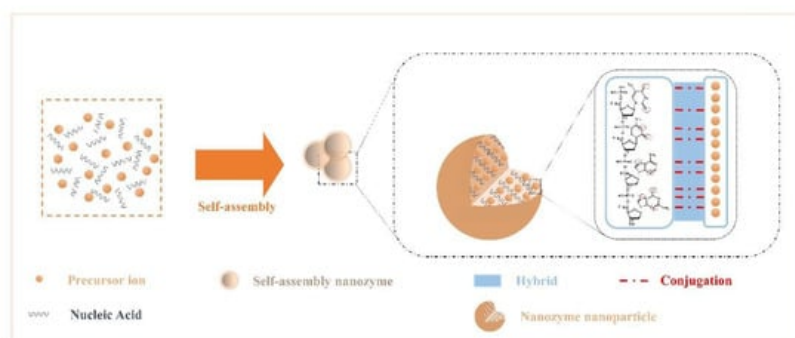


Figure 1. Crosstalk from self-assembled nanozymes: noncovalent interactions that occur during the assembly of NPs and nucleic acids.

2.2. Irreversible Binding Nanozyme

Nucleic acids have a complex skeletal structure composed of bases, phosphate groups, and ribose, so they have a wealth of modification sites^{[60][61]}, while NPs have the characteristics of many surface active sites, strong adsorption, and a high density of valence electrons. Researchers have made use of their rich properties, modified them, and accomplished the irreversible combination of the two components. Many modification bridges have been used to promote binding between NPs and nucleic acids (Figure 2), for example, $-\text{NH}-\text{SiO}_2-$, -biotin-streptavidin-, $-\text{NH}-\text{COOH}-$, $-\text{C}-\text{NH}-$, $-\text{S}-$, -magnetic bead-, and $-\text{NH}-$ ^{[62][63][64][65][66][67][68]}. Binding at the interface perfectly merges the advantages of the two. Additionally, irreversible chemical modification may affect an NP's characteristics and limit the number of connections of nucleic acids. In summary, functional nanozymes have both the enzyme activity of NPs and the target recognition function of nucleic acids, producing the desired interface effect, which can be applied to biochemical energy conversion events.

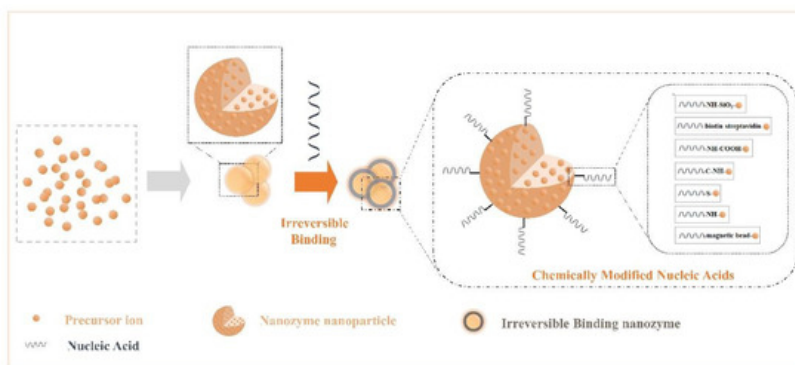


Figure 2. Crosstalk from irreversible binding nanozymes: creating irreversible binding nanozymes based on synthetic materials (such as chemically modified nucleic acids).

2.3. Reversible Binding Nanozyme

Nucleic acids have parallel stacked bases, polymerized anionic phosphate backbones, sugar rings, and active sites composed of large grooves and small grooves formed by double-stranded structures^[25], and these features allow nucleic acids to combine/separate with nanozymes with high specific surface areas and rich surface chemical morphologies, as expected in various analytical events based on competitive binding mechanisms^[69]. In general, the addition of nucleic acids to the nanozyme reaction system increases the interface components of the NPs, dramatically increases the free energy of the interface with the NPs, and changes the structure-related properties of the interface, such as ionic valence and electronic transfer. Moreover, it can be formed into a reversible binding nanozyme, which preserves the characteristics of nucleic acids and NP as much as possible. Here, we list the general influence of nucleic acid on NPs (Figure 3) as changes in the steric hindrance effect, changes in the number of active sites, changes in the dispersion of NPs, changes in the structure of NPs (such as oxygen vacancies), and changes in substrate activity in producing highly active substances (such as hydroxyl radicals).

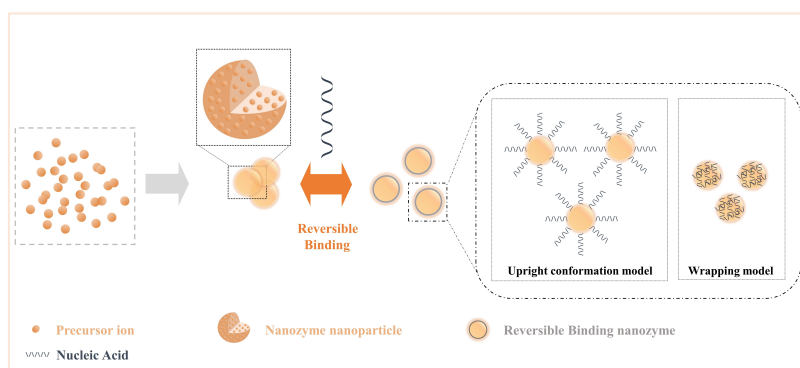


Figure 3. Crosstalk from irreversible binding nanozymes and reversible binding nanozymes allow nucleic acids to combine/separate with nanozymes by the effect of intermolecular forces. Reprinted from reference^[69].

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