Recent advances in the construction of nanozyme-based logic gates

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Abstract Nanozymes, nanomaterials with enzyme-like activity, have been considered as promising alternatives of natural enzymes. Molecular logic gates, which can simulate the function of the basic unit of an electronic computer, perform Boolean logic operation in response to chemical, biological, or optical signals. Recently, the combination of nanozymes and logic gates enabled bioinformation processing in a logically controllable way. In the review, recent progress in the construction of nanozyme-based logic gates integrated with their utility in sensing is introduced. Furthermore, the issues and challenges in the construction processes are discussed. It is expected the review will facilitate a comprehensive understanding of nanozyme-based logic systems.

Keywords Nanozyme, Logic gate, Sensor, Regulation, Input

INTRODUCTION

The computer processes digital information and performs logical operation based on silicon-circuitry and logic gates. Electrical signals are used as inputs and outputs of logic gates which follow the laws of Boolean algebra. The absence and presence of electrical signals are defined as 0 and 1, respectively. Boolean logic gates produce binary outputs 0/1 upon treatment with binary inputs 0/1, corresponding to AND, OR, INHIBIT, NAND, NOR, XOR, XNOR, *etc.* Originating from the requirements of computer miniaturization and increase of function density, Boolean logic operations have been realized on the molecular levels (Andreasson and Pischel 2010; de Silva and McClenaghan 2004; Erbas-Cakmak *et al.* 2018; Pu *et al.* 2014b). In general, molecular logic gates are implemented based on molecular switching or chemical reaction. Various chemical and physical signals serve as inputs and as outputs. In the past few decades, inspired by the biocatalytic reactions in biological process, great

efforts have been made in construction of logic gates and computing components based on natural enzymes (Katz and Privman 2010; Lin *et al.* 2012). Nanozyme, the nanomaterial with enzyme-like properties, has attracted great attention in recent years (Huang *et al.* 2019; Wei and Wang 2013; Wu *et al.* 2019a). Compared with natural enzyme, nanozyme presented the advantages of easy preparation and modification, high stability, duration under harsh conditions, size/morphology/composition dependent activity, and low cost. As an alternative, nanozymes show potential application in environment, biomedicine, and food industry (Huang *et al.* 2018, 2019; Wang *et al.* 2016). Nanozymes have also been used as computing elements to mimic the functions of logic gates. On the one hand, nanozyme-based logic gates borrowed some ideas from natural enzyme-based biological information processing. On the other hand, the properties of nanomaterials themselves made their performance superior to that of natural enzymes. However, so far, there is no review on the nanozyme-based logic gates. In this review, we illustrated how Boolean logic gates were realized using

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nanozymes. Meanwhile, the applications of nanozyme-based logic gate in sensing were introduced. Furthermore, the existing issues and challenges in the field were discussed.

TYPES OF NANOZYMES USED FOR CONSTRUCTION OF LOGIC GATES

Current studies on logic gates focus on oxidoreductase, including oxidase, peroxidase, and catalase (Table 1). Oxidase catalyzes the oxidation of a substrate with the assistance of O_2 to produce oxidized product and $H_2O/H_2O_2/O_2^-$. Glucose oxidase can catalyze glucose to yield gluconic acid and H_2O_2 in the presence of O_2 . Gold nanoparticles (AuNPs) are the most studied mimic of glucose oxidase (Zhang *et al.* 2018). Peroxidase can oxidize a substrate with the help of H_2O_2 . Carbon-based nanomaterials (graphene, carbon nanotubes, *etc.*) and metal oxides (Fe_3O_4 , CuO, V_2O_5 , *etc.*) have been found to possess excellent peroxidase-like properties (Andre *et al.* 2011; Gao *et al.* 2007; Song *et al.* 2010; Wang *et al.* 2018b). Catalase decomposes H_2O_2 into H_2O and oxygen. Some metal oxides were reported to present catalase-like activity (Mu *et al.* 2013). Moreover, some nanozymes possess multiple enzyme-like activities (Wu *et al.* 2019a). For instance, AuNPs with the diameter of 13 nm have peroxidase-, oxidase-, and catalase-like activities. Taking advantage of these nanozymes, a series of logic gates were constructed.

LOGIC GATES BASED ON NANOZYMES

Different from conventional silicon-based digital elements which use electrical signals as inputs and outputs, the nature of the input and output signals of molecular logic gates is different. For nanozyme-based logic gates, nanozymes, substrates, external substances, and physical stimuli could serve as logic unit or inputs. The final absorbance or fluorescence of reaction products serves as outputs. In this review, according to the composition of logic gates, nanozyme-based logic gates are categorized into two classes: nanozymes themselves as inputs and regulation factors of nanozymes as inputs.

Nanozymes as inputs to construct logic gates

Inspired by intracellular enzyme-participated redox balance system, Huang *et al.* constructed an AND gate by employing two artificial enzymes: Au nanoparticle-doped mesoporous silica nanoparticles (SiO_2 -Au) and G-quadruplex-hemin DNAzyme as inputs (Huang *et al.* 2017). SiO_2 -Au with glucose oxidase-like activity could catalyze glucose to generate gluconic acid and H_2O_2 . DNAzyme presented peroxidase-like activity, through which H_2O_2 could be transformed to $\cdot OH$ and ABTS could be oxidized to $ABTS^{+}$. The cascade reaction produced colored product when both the inputs existed, corresponding to AND gate (Fig. 1). Both G-quadruplex-hemin DNAzyme and V_2O_5 nanowire could mimic peroxidase to catalyze the same reaction.

Table 1 The representative materials for construction of logic gates

Enzyme-like activity	Nanozymes	References
Glucose oxidase	Au nanoparticle-doped mesoporous silica nanoparticles (SiO_2 -Au)	Huang <i>et al.</i> (2017)
	AuNPs	Zhan <i>et al.</i> (2014)
Peroxidase	V_2O_5 nanowire	Huang <i>et al.</i> (2017)
	CuO:Graphene nanosphere (CuO:GNS)	Sharma and Mobin (2017)
	AuNPs	Lien <i>et al.</i> (2013, 2014)
	bimetallic nanoparticle PtAuNPs	Lin <i>et al.</i> (2017)
	Hemin-graphene (HG) hybrid nanosheet	Lin <i>et al.</i> (2014)
	Copper (II)-based metal-organic frameworks (Cu-MOFs)	Wang <i>et al.</i> (2018a)
	histidine-protected gold nanoclusters (His-AuNCs)	Chen <i>et al.</i> (2019)
	NH_2 -MIL-101 MOFs	Li <i>et al.</i> (2019)
Catalase	AuNPs	Lien <i>et al.</i> (2013)
	Co_3O_4 nanoparticles	Han <i>et al.</i> (2018)
Oxidase	AuNPs	Lien <i>et al.</i> (2013)
	Manganese dioxide nanosheet (MnO_2 NS)	Fan <i>et al.</i> (2017)
	9-mesityl-10-methylacridinium ion (Acr^{+} -Mes)	Du <i>et al.</i> (2018)
	MnO_2 nanosheets	Zhao <i>et al.</i> (2018)

Fig. 1 Scheme (A) and truth table (B) of nanozyme-based AND gate. C Absorbance and photograph of reaction product after being treated with the combination of different inputs. Reproduced with permission of Wiley-VCH (Huang *et al.* 2017)

Based on it, an OR gate was constructed. MnO_2 could catalyze the decomposition of H_2O_2 to produce H_2O and O_2 . Without H_2O_2 , DNAzyme could not transform ABTS to ABTS^{++} . Combining MnO_2 and DNAzyme, an INHIBIT gate was built.

Sharma *et al.* found that CuO:Graphene nanosphere (CuO:GNS) composite could mimic peroxidase (Sharma and Mobin 2017). Phenol coupled with 4-aminoantipyrine (4-AAP) in the presence of H_2O_2 , the main product of oxidation of cholesterol by cholesterol oxidase, could be catalyzed by CuO:GNS to yield a red colored product. The reaction was employed for colorimetric sensing of H_2O_2 and cholesterol. Furthermore, using CuO:GNS and cholesterol as inputs, an AND gate was constructed.

Logic systems based on the regulation of the catalytic activities of nanozymes

Metal ions, biomolecules, pH, and light illumination could regulate the catalytic activity of nanozymes. These external substances and physical stimuli were used as inputs to construct logic gates.

Metal ions

The catalytic activity of nanozyme can be activated or inhibited by depositing metal ions. Taking advantage of this property, Lien *et al.* constructed multiplex logic operations (OR, AND, INHIBIT, and XOR gates) through regulation of the enzyme-like activity of AuNPs upon addition of different metal ions (Lien *et al.* 2013). AuNPs (13 nm) exhibited multiple enzyme-like activities, including peroxidase-, oxidase-, and catalase-like activity. The oxidase-like activity of AuNPs could be enhanced by co-deposition of $\text{Pt}^{4+}/\text{Hg}^{2+}$, resulting from $\text{Pt}^{4+}/\text{Hg}^{2+}$ -induced high oxidative catalytic ability and

minimizing deactivation. An AND gate could be constructed using the oxidase-like activity of AuNPs and $\text{Pt}^{4+}/\text{Hg}^{2+}$ ions as inputs. The catalase-like activity of AuNPs could be enhanced by Hg^{2+} , Bi^{3+} , and their mixture, accompanied by stronger surface plasmon resonance (SPR) absorption. The catalase-like activity of AuNPs and $\text{Hg}^{2+}/\text{Bi}^{3+}$ as inputs was used to construct an OR gate. The peroxidase-like activity of AuNPs was very low due to the low variation of surface metal oxidation states. Pb^{2+} adsorbed on the surface enhanced the peroxidase-like activity, resulting from various valance (oxidation) states of the Pb ($\text{Pb}^{2+}/\text{Pb}^0$) and Au (Au^+/Au^0). Hg^{2+} presented slight effect on the peroxidase-like activity due to its low oxidation state. The competition between Pb^{2+} and Hg^{2+} ions for AuNPs presented an INHIBIT logic operation, in which the peroxidase-like activity of AuNPs served as the output. The strong Au-Ag metallophilic interactions and ease of reduction of Ag^+ resulted in the enhanced peroxidase-like activity. In the presence of both Ag^+ and Bi^{3+} , the formation of stable Ag/Bi alloys decreased surface potentials and active sites, reducing the activity. The regulation of peroxidase-like activity of AuNPs by Ag^+ and Bi^{3+} corresponded to a XOR gate. Furthermore, due to the stable surface lattice structures and/or low surface energy of Pt^{4+} and Pb^{2+} , the peroxidase-like activity of AuNPs was low when one of them was presented. The co-deposition of Pt^{4+} and Pb^{2+} caused enhancement of the peroxidase-like activity. It might be attributed to that the formation of alloys with various valence states led to increased surface potentials and active sites. Based on it, the same group constructed AND gate using $\text{Pt}^{4+}/\text{Pb}^{2+}$ ions and the peroxidase-like activity of AuNPs in the presence of $\text{S}_2\text{O}_3^{2-}$ (Lien *et al.* 2014). Besides, enhanced activity could be achieved by Bi^{3+} due to the various valence (oxidation) states of Bi^{3+} and Au (Au^+/Au^0). Upon introduction of Hg^{2+} , the surface of AuNPs was passivated, presented low activity. Thus, an INHIBIT gate using $\text{Bi}^{3+}/\text{Hg}^{2+}$ and peroxidase-like activity of AuNPs was constructed. An integrated logic circuit was also constructed for the visual detection of Pb^{2+} and Hg^{2+} (Fig. 2).

Biomolecules

Owing to easy synthesis and modification, robust physicochemical nature, tunable polymorphic structure, and specific recognition, nucleic acids are integrated with nanomaterials to construct logic gates (Pu *et al.* 2014b). Recently, nucleic acids were used to manipulate catalytic behavior of nanozymes (Liu and Liu 2015; Zheng *et al.* 2011). However, the phenomenon and explanation for the regulation mechanism are

Fig. 2 **A** Three-input/three-output integrated logic circuit. **B** Images of solutions of AuNPs/Bi³⁺/H₂O₂ (upper) and AuNPs/Pt⁴⁺/S₂O₃²⁻/H₂O₂/AR (bottom) treated with nothing (a), Pb²⁺ (b), Hg²⁺ (c), and Pb²⁺/Hg²⁺ (d). **C** Truth table. Reproduced with permission of American Chemical Society (Lien *et al.* 2014)

contradictory. Nucleic acids could enhance the activity of nanozymes since DNA absorbed on the surface of nanozyme could increase substrates binding through electrostatic interaction (Liu and Liu 2015). In the other case, nucleic acids suppressed the catalytic reaction by absorbing on the surface of nanozyme to block the active sites (Zheng *et al.* 2011). Although the mechanism is not yet comprehended, nucleic acid-regulated nanozyme-based logic gates were constructed.

AuNPs can mimic the catalytic activity of glucose oxidase (GOx). Nucleic acids absorbed on the surface of AuNPs change the surface properties, thus affecting the catalytic activity. Zhan *et al.* investigated the influence of pH-sensitive nucleic acid strands on AuNPs and demonstrated pH-induced conformational change of DNA strands endows AuNPs with different catalytic efficiency (Zhan *et al.* 2014). Using pH and different DNA strands as inputs, a series of logic gates (AND, XNOR, and NOT) were designed.

Nucleic acids can act as a linker to control assembly of nanoparticles through programmed Watson–Crick base pairing. Taking advantage of the properties of

nucleic acid, Lin *et al.* developed a nanozyme-based bio-barcode fluorescence assay (Lin *et al.* 2017). The complementary DNA sequence of HIV (CHIV) was split into two segments, CHIV1 and CHIV2. Then they were modified on the surface of bimetallic nanoparticle PtAuNPs and magnetic nanoparticles (MNPs), respectively. Upon addition of HIV DNA, PtAuNPs and MNPs would be linked together via hybridization of HIV DNA with the two segments. After magnetic separation, amplex red (AR) and H₂O₂ were added. PtAuNPs presented peroxidase-like activity and could catalyze non-fluorescent substrate AR into fluorescence product. The concentration of HIV DNA could be quantified by measuring the change of fluorescence induced by the catalytic reaction. Similarly, HCV DNA could also be detected based on the same principle. Using HIV and HCV DNA as inputs, an OR logic gate was constructed (Lin *et al.* 2017). Furthermore, cascaded INHIBIT-OR logic gate was integrated with a biosensor to distinguish individual HIV and HCV genes in mixture. The CHIV as mask agent could depress signal change caused by HIV DNA. An INHIBIT gate could be constructed using CHIV

and HIV DNA as inputs. By further integrating with the OR gate, HIV and HCV DNA could be distinguished.

Nucleic acid can also drive assembly of nanoparticles through specific recognition. Based on the high affinity between aptamer and protein, Lin *et al.* used protein-mediated aggregation of DNA-absorbed hemin-graphene nanosheet to build colorimetric protein assay and logic gates (Fig. 3) (Lin *et al.* 2014). Hemin-graphene (HG) hybrid nanosheet possesses intrinsic peroxidase-like catalytic activity. The self-assembly of the

hybrid nanosheet can cause precipitation after centrifugation. The supernatant presented low catalytic activity due to the low amount of the hybrid nanosheet. The interaction between proteins and aptamers absorbed on the surface of hemin-graphene nanosheet led to the cross-linking aggregation, thus changing the colorimetric signal of the supernatant. Thrombin and PDGF-BB as model analytes could be detected with high sensitivity. Furthermore, using thrombin and PDGF-BB as inputs and aptamer-absorbed HG as logic unit, an OR

Fig. 3 OR (A) and INHIBIT (B) gates using protein-mediated aggregation of DNA-absorbed hemin-graphene nanosheet. Reproduced with permission of American Chemical Society (Lin *et al.* 2014)

gate could be constructed. In the presence of thrombin, PDGF-BB, and their mixture, sandwich structure of HG/DNA/protein could be formed through specific binding between proteins and their aptamers, leading to reduction of catalytic activity of centrifugal supernatant. By changing DNA sequence, an INHIBIT operation could be realized. Two DNA strands absorbed on the surface of HG could hybrid to cause aggregation of HG. Since one of them was aptamer of thrombin, the addition of thrombin could inhibit their hybridization and aggregation of HG. However, PDGF-BB not only did not disturb the hybridization, but also caused formation of sandwich structure of HG/aptamer/PDGF-BB. Adding both thrombin and PDGF-BB still resulted in aggregation of HG. The output signals corresponded to an INHIBIT gate.

Glutathione (GSH), tripeptide containing a thiol group, plays crucial roles in several biological processes. GSH could change the catalytic activity of nanozyme by reacting with nanozyme. Manganese dioxide nanosheet (MnO_2 NS) was regarded as an oxidase mimic and fluorescent Scopoletin (SC) and nonfluorescent AR could serve as the substrates of MnO_2 NS. Fan *et al.* found MnO_2 NS could quench the fluorescence of SC and enhance the fluorescence of AR through oxidation reaction (Fan *et al.* 2017). However, GSH could reduce MnO_2 to Mn^{2+} , leading to the loss of oxidase-like activity. The fluorescence of SC increased while the fluorescence of AR decreased. Based on the dual-signal response, a ratiometric sensor for detection of GSH was constructed. The output signals corresponded to an INHIBIT gate cascade with a 1 to 2 decoder (Fig. 4).

Some biomolecules could interact with composition of nanozymes and block their catalytic activity. Copper (II)-based metal-organic frameworks (Cu-MOFs) showed peroxidase-like activity. The catalytic activity of Cu-MOFs was greatly inhibited by pyrophosphate (PPi) due to the ultrastrong binding between Cu^{2+} and PPi. Alkaline phosphatase (ALP) could hydrolyze PPi into orthophosphates (Pi), which have low affinity to Cu^{2+} . This led to the recovery of catalytic activity of Cu-MOFs, allowing visual analysis of ALP. Taking advantage of the activity change induced by PPi and ALP, Wang *et al.* constructed an IMPLICATION logic gate (Wang *et al.* 2018a). Based on the similar principle, Chen *et al.* used histidine-protected gold nanoclusters (His-AuNCs) with peroxidase-like activity constructed colorimetric sensor for phosphate-containing metabolites and logic gate (Chen *et al.* 2019). Besides PPi, other phosphate-containing metabolites including adenosine triphosphate (ATP) and adenosine diphosphate (ADP) could hamper the catalytic activity of His-AuNCs and be hydrolyzed by ALP. Using the three phosphate-containing metabolites as inputs and His-AuNCs as logic unit, a NOR gate was built. By further adding ALP as input, a NOR+IMPLICATION gate was constructed.

NH_2 -MIL-101 MOFs as a peroxidase-like nanozyme catalyze oxidation of o-phenylenediamine (OPD) by H_2O_2 to generate fluorescent 2,3-diaminophenazine. In the catalytic reaction, the inherent fluorescence emission at 456 nm (F_{456}) of NH_2 -MIL-101 MOFs was quenched, while the fluorescence of catalytic product 2,3-diaminophenazine at 556 nm (F_{556}) increased. The catalytic activity could be inhibited by PPi through binding of PPi and Fe center, and then restored by acid

Fig. 4 Scheme of the cascade logic circuit (INHIBIT-1 to 2 Decoder). Reproduced with permission of American Chemical Society (Fan *et al.* 2017)

phosphatase (ACP). Based on the PPI/ACP-mediated fluorescence changes at different wavelengths, Li *et al.* constructed a ratiometric fluorescence sensor for ACP and an INHIBIT gate cascade with 1–2 decoder (Li *et al.* 2019).

Light

Light as modulator of nanozyme attracts great attention owing to its high temporal and spatial precision, and ease in controlling the intensity and wavelength (Huang *et al.* 2019; Wu *et al.* 2019b). Different from the most nanozymes who showed the “on” state under normal conditions, photo-responsive nanozymes presented catalytic activity in the presence of light. For example, upon visible light illumination, 9-mesityl-10-methylacridinium ion (Acr^+ -Mes) presented oxidase-like activity for catalytic oxidation of 3,3',5,5'-tetramethylbenzidine (TMB). Du *et al.* constructed visual logic gates (AND, INH, and NOR) based on the catalytic reaction (Fig. 5) (Du *et al.* 2018). An AND logic operation could be performed using TMB as logic unit

and Acr^+ -Mes and light illumination as inputs. L-cysteine and L-glutathione could restrain the photo-oxidation of TMB by Acr^+ -Mes. Using TMB and light illumination as logic unit and L-cysteine and Acr^+ -Mes as inputs, an INH gate was constructed. Using TMB, light illumination and Acr^+ -Mes as logic unit and L-cysteine and L-glutathione as inputs, an OR gate was constructed. The system could be used for colorimetric detection of L-cysteine and L-glutathione.

pH

One nanozyme can show different types or catalytic efficiency at different pH. For example, Fe_3O_4 nanoparticles exhibit peroxidase-like activity at acidic pH while exhibit catalase-like activity under neutral environment. Also, the oxidase-like activities of MnO_2 nanosheets are pH switchable and reversible. At acidic condition, MnO_2 nanosheets show oxidase-like activity. When pH becomes alkaline, the activity decreases due to the transformation into hexagonal Na_4MnO_4 . Based on the phenomenon, Zhao *et al.* constructed a three-input molecular majority

Fig. 5 Scheme, output results, and truth table of logic gates based on light-activatable Acr^+ -Mes with an oxidase-like activity. Reproduced with permission of American Chemical Society (Du *et al.* 2018)

logic gate with a single-vote veto function (Fig. 6). The alkaline TMB solution served as the logic unit, and the MnO_2 nanosheets, H^+ , and H_2O_2 served as inputs. Only when two or more inputs were added, the oxidation of TMB to oxTMB occurred, which corresponded to a three-input majority logic function. However, in the strong basic condition, the oxidation of TMB was inhibited since the oxidation capacity of H_2O_2 and oxidase-like activity of the MnO_2 nanosheets remarkably reduced. Thus, NaOH as the other input could perform a single-vote veto function. In addition, using H^+ and TMB as logic unit, and H_2O_2 , MnO_2 , and NaOH as inputs, an OR-INH cascade logic circuit could be realized (Zhao *et al.* 2018).

Multiplex factors

In general, definitive logic unit and inputs output fixed logic signal. It is difficult and significant to realize logic conversion of sole logic system. Inspired from nature in

which organisms can be programmed into different forms in different environment, Han *et al.* realized the transformation of logic functions using definitive logic unit and inputs (Fig. 7) (Han *et al.* 2018). The metal ions could alter the catalase-like activity of Co_3O_4 nanoparticles in type- and pH-dependent manners. Only by changing pH of the logic systems, switchable logic operations could be realized. For the system using Co_3O_4 as logic unit and $\text{Hg}^{2+}/\text{Fe}^{3+}$ as inputs, an OR gate was constructed at pH 10.0. When the pH was changed to 7.0, the system performed an INHIBIT function. Using Ca^{2+} and Mn^{2+} as inputs, INHIBIT and AND gates were obtained at pH 10.0 and pH 7.0, respectively.

SUMMARY AND PERSPECTIVES

In summary, recent development of nanozyme-based logic gates was summarized. Despite this valuable

Fig. 6 **A** Scheme of the three-input majority logic gate based on pH-modulated oxidase-like activity of MnO_2 nanosheets. **B** UV-vis absorption spectra (a), photographs (b), absorbance intensities at 652 nm (c), and truth table (d) of the three-input majority logic gate with different combinations of the inputs. Input 1, input 2, and input 3 were H_2O_2 , MnO_2 , and H^+ , respectively. The absence and presence of the inputs represented 0 and 1, respectively. I–VIII: (0, 0, 0), (1, 0, 0), (0, 1, 0), (0, 0, 1), (1, 0, 1), (1, 1, 0), (0, 1, 1), (1, 1, 1); IX: NaOH and (1, 1, 1). Reproduced with permission of Royal Society of Chemistry (Zhao *et al.* 2018)

Fig. 7 Influence of different metal ions on the catalase-like activity (A, B) and electrocatalytic activity (C, D) of Co₃O₄ nanoparticles at pH 7.0 and pH 10.0. E Scheme of pH-switchable logic conversion. F OR/INHIBIT-switchable logic gate. G INHIBIT/AND-switchable logic gate. Reproduced with permission of Wiley-VCH (Han *et al.* 2018)

progress, nanozyme-based logic gates remain in its early stage. Some challenges still exist in this field.

- (1) The nanozymes used for construction of logic gates are mainly mimics of oxidoreductase, such as peroxidase, oxidase, and catalase. This is limited by the type of nanozymes themselves. With the development of new nanozymes which can catalyze other types of reaction beyond redox reactions, the range of selection will expand. Moreover, nanozymes can be combined with natural enzymes or other chemical reactions to achieve logic functions.
- (2) Most of the current nanozyme-based logic gates could only implement simple logic operations, such as AND, OR, and INHIBIT. Constructing complex logic systems to realize advanced information processing is less explored. This can be attributed to the high complexity of circuit design. For example, different nanozymes have their own optimal reaction conditions. When they are in a uniform reaction system, they may show low

catalytic efficiency and unsatisfactory stability, thus limiting the realization of logic functions.

- (3) Nanozymes could interact with a series of biomolecules, including proteins, nucleic acids, peptides, and even cells (Qiu *et al.* 2018; Wang *et al.* 2018c). The nonspecific binding of these biomolecules could interfere with the catalytic activity of nanozymes. Therefore, it is necessary to consider this factor in the design of logic gates integrated with sensors and diagnostic reagents.
- (4) Some external substances could regulate the catalytic activity of nanozymes. Although they acted as inputs to construct logic gates, the mechanism of their regulation effect on nanozyme activity has not been fully understood.
- (5) The reset capability is a key factor for the reusability of logic gates. However, it is difficult to realize the reset of these nanozyme-based logic systems to the original state since the inputs are hard to be separated from the reaction solution. Moreover, the accumulation of by-product may affect the performance of logic gates. Therefore,

design of resettable nanozyme-based logic systems is a crucial study for practical applications.

- (6) An important goal of constructing molecular logic systems is the ultimate translation for practical applications. However, due to limited complexity of molecular logic gates, it is difficult to compete with electronic computers. We had to think about the issue with a different view. The molecular logic gates can simplify the bioinformation and output the diagnosis or detection result as “0” or “1,” which means the absence or presence of disease and analytes. This provides another avenue for applications of molecular logic gates. Several molecular logic gates have been integrated with various functions, such as drug delivery/release, bioimaging, disease diagnosis, regulating the expression of specific disease genes, and biosensing (Pu *et al.* 2014a; Ran *et al.* 2018). However, most nanozyme-based logic gates were only integrated with biosensors. Nanozymes have been used for cancer treatment, inflammation elimination, and antibacterial therapy based on their catalytic properties in response to the physiological environment (Huang *et al.* 2016; Qiu *et al.* 2020; Wang *et al.* 2018d). The logic gates coupling with these biological applications will offer future intelligent diagnosis and therapy.
- (7) The current nanozyme-based logic functions were performed in solution. Absorbance and fluorescence signals were used as outputs. The characteristics of nanomaterials enable them to be modified on the interface to realize catalytic reactions, which can offer transduction of the biochemical output into electronic or other signals. This may provide an opportunity for the fabrication of portable logic devices.

Nonetheless, the advantages of nanozymes still offer tremendous opportunities for biologic information processing. With further development of nanomaterials with enzyme-like activity, more sophisticated and useful logic systems will be designed and built.

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Compliance with Ethical Standards

Conflict of interest Fang Pu, Jinsong Ren, and Xiaogang Qu declare that they have no conflict of interest.

Human and animal rights and informed consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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