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# On the Specificity of Nanozymes: A Perspective

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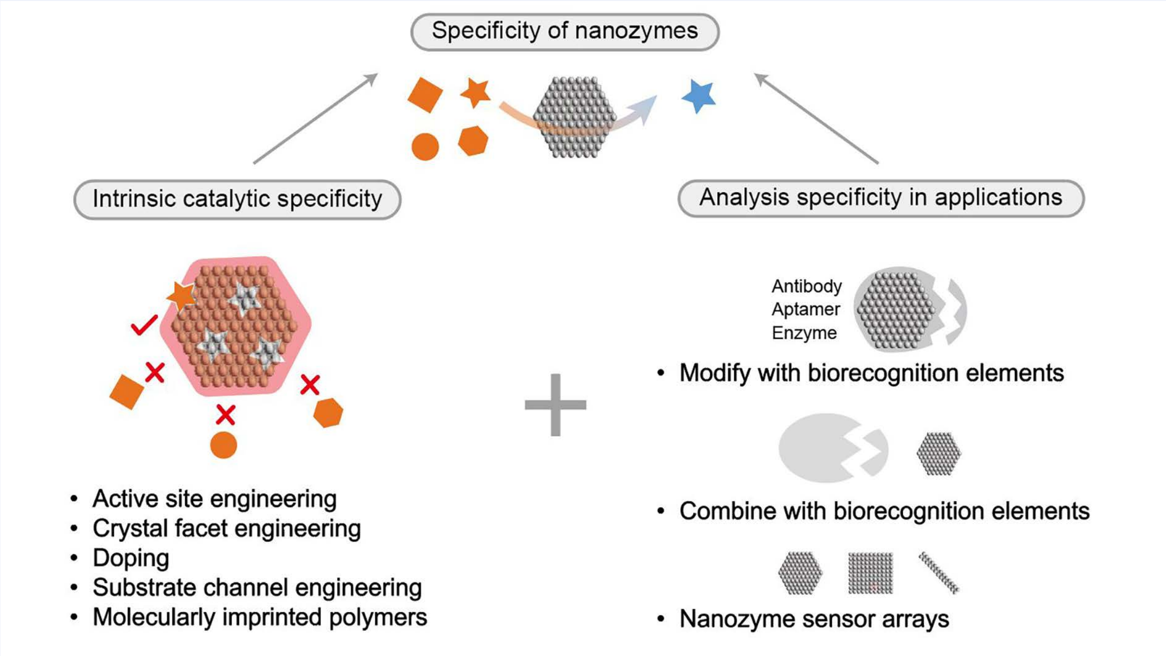
## Keywords

Nanozymes | Enzymes | Catalytic activity | Specificity | Perspective

## Comprehensive Summary



We have compiled eight promising strategies for enhancing the specificity and selectivity of nanozymes, as depicted in the comprehensive summary above. Enzymes exhibit intricate and sophisticated structures, including substrate channels and active sites, which can inform the design of nanozymes. Replication of these structural features and the application of facet engineering/doping techniques can significantly enhance the catalytic specificity of nanozymes. Alternatively, the use of Molecularly Imprinted Polymers (MIPs) to coat nanozymes represents an effective approach to impart substrate specificity. Furthermore, several straightforward stopgap strategies have been devised to improve nanozyme specificity for analytical applications, such as the integration of biorecognition elements and nanozyme sensor arrays through surface modification.

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Combine enzymes for selective bioassays

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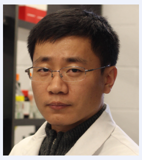
Modulate the catalytic selectivity of nanozymes by facet engineering



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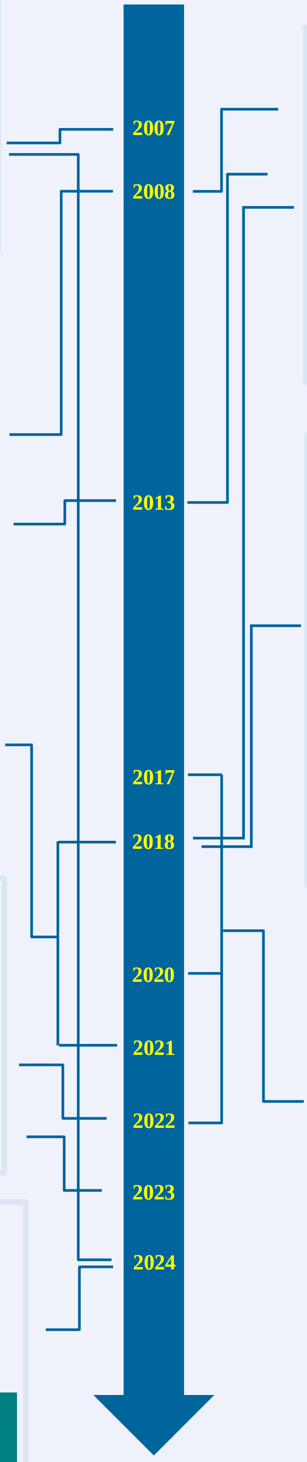
Perspective on specificity of nanozymes



*Stories to be Continued ...*

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General definition of nanozymes
Modulate catalytic selectivity of carbon nanozymes using a N-doping strategy
Combine enzymes for selective bioassays

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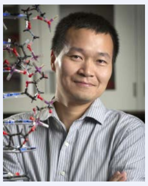
Achieve substrate specificity by introducing substrate channels within nanozymes



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Endow a nanozyme with substrate specificity by molecular imprinted polymers (MIPs)

## Contents

## 1. Introduction

Enzymes are catalytic proteins or nucleic acids. They catalyze biological reactions with high efficiency and specificity. However, because of their proteinaceous and nucleic acid-based nature, enzymes have several intrinsic limitations, such as low stability, high cost, and storage difficulty. These limitations have hindered their broad applications. To address these limitations, various artificial enzymes (also called "enzyme mimics") have been developed.[1-2] In 2007, Yan *et al.* discovered that magnetic $Fe_3O_4$ nanoparticles (NPs) had peroxidase-like activity.[3] Since then, thousands of nanomaterials have been found to exhibit various enzyme-like activities. Currently, these "nanomaterials with enzyme-like characteristics" are collectively called "nanozymes".[1-2,4-8]

Nanozymes combine the merits of functional nanomaterials and conventional artificial enzymes (Figure 1). They not only possess rich surface chemistry, mild catalytic conditions, multiple functionalities, and tunable activities, but they are also mass-produced, stable, cost effective, and recyclable. Therefore, since their discovery, nanozymes have been explored for wide applications, ranging from bioanalysis and bioimaging to therapeutics, environmental protection, and agriculture.[9-18]

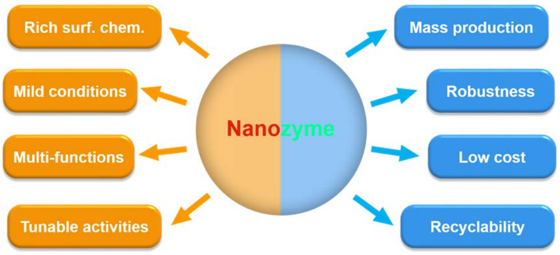


**Figure 1** Features of nanozymes. Nanozymes combine the merits of functional nanomaterials and conventional artificial enzymes.

Unlike enzymes, which generally exhibit strong substrate-specific activities, nanozymes may possess multiple enzyme-like activities. While these multiple enzyme-like activities enable nanozymes to engage in self-cascade reactions, the lack of substrate specificity may hinder the development of selective bioassays and other applications. Of note, researchers in the nanozyme community have recognized this challenge and have developed several strategies to tackle it. These strategies can be roughly classified into two categories: one focuses on the intrinsic specificity of nanozymes, while the other concentrates on analysis specificity from an application aspect. From a structural perspective, the specificity of enzymes originates from their unique active sites and substrate channels, which enable selective substrate binding and subsequent catalysis. Consequently, drawing inspiration from the active sites and substrate channels of enzymes, simulating these enzymatic structures within nanozymes or accessing the interaction between nanozymes and substrates may enable selective binding and catalysis of the substrate by nanozymes. Nevertheless, nanozymes with intrinsic specificity are still rare. From an application standpoint, particularly in analysis, it is important to improve the specificity of nanozymes by employing external attributes. Therefore, in this perspective, we aim to summarize strategies for engineering nanozymes with intrinsic catalytic specificity and for conferring analysis selectivity through the use of external attributes. Furthermore, we provide our insights into future directions.

## 2. Strategies to Develop Nanozymes with Intrinsic Catalytic Specificity

In early studies, five strategies have been developed to address the intrinsic catalytic specificity of nanozymes. They are discussed below.

### 2.1. Active site engineering

Enzymes possess well-defined active sites, which are of great importance to catalytic specificity. An enzymatic active site usually has a unique three-dimensional geometry, coordination environment, and well-positioned cofactors. By mimicking these unique features of enzymatic active sites, it is possible to design nanozymes capable of selective recognition of substrate.[19-23] As shown in Figure 2, simulating an iron porphyrin ring and consequently embedding iron atoms into a defective $Fe\text{-}N_4$ structure achieved nearly a 10-fold increase in selective adsorption and activation of $H_2O_2$ compared to the $C\text{-}N_4$ framework, which significantly improved the catalase (CAT)-like activity of defective $Fe\text{-}N_4$.[24] Additionally, the trinuclear Cu active site structure of the metal–organic framework-818 (MOF-818) nanozyme, resembling the active site of a catechol oxidase, exhibited selective oxidation of diphenols.[25]

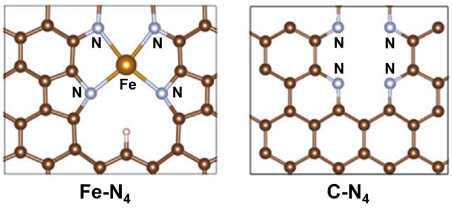


**Figure 2** Defective coordination structure of active sites to endow $Fe\text{-}N_4$ nanozyme with specific CAT-like activity ($C\text{-}N_4$ nanozyme as a comparison). Reprinted with permission from ref. [24]; copyright (2022) Wiley Online Library.

### 2.2. Substrate channel engineering

Besides active sites, substrate channels also play a critical role in enzyme specificity. These channels possess not only specific sizes and shapes but also hydrophobic and hydrophilic regions, enabling specific substrate binding and transportation. Consequently, by introducing substrate channels within nanozymes, substrate specificity could also be achieved. As shown in Figure 3, by etching PtNi nanoparticles, isolated substrate channels were obtained, which significantly improved the electrocatalytic activity for the oxygen reduction reaction.[19] The substrate channel engineering strategy has subsequently been demonstrated in other nanozymes.[20-23]

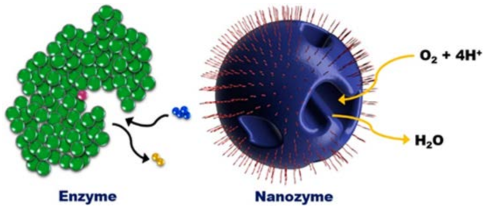


**Figure 3** Substrate channel engineering to endow nanozymes with specificity. Reprinted with permission from ref. [19]; copyright (2018) American Chemical Society.

### 2.3. Crystal facet engineering

Crystal facet engineering can modify the structure of the exposed surface and active site of nanozymes. Therefore, it is feasible to modulate the catalytic selectivity of nanozymes by facet engineering. A recent study compared the CAT-like activity of cube-, rod-, and octahedron-shaped $CeO_2$ nanozymes. The cube enclosed by the (100) facet exhibited the highest CAT-like activity, while the octahedron enclosed by the (111) facet demonstrated the lowest CAT-like activity.[26]

A more recent study further validated the effectiveness of facet engineering (Figure 4).[27] For $CeO_2$ nanozymes, the $Ce_{6c(100)}$ sites of the cube and the $Ce_{7c(111)}$ sites of the octahedron exhibited specific peroxidase- and haloperoxidase-like activity, respectively. Facet engineering essentially modulates the coordination and electronic structures of Ce sites, thereby regulating the hydrogen peroxide activation pathway on various facets.

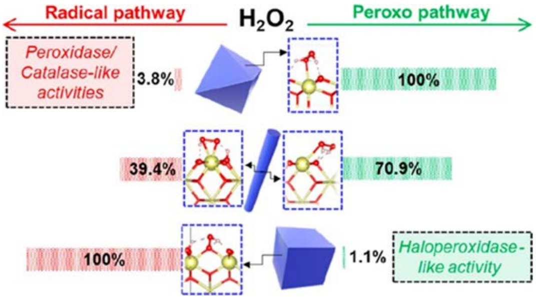


**Figure 4** Modulate enzyme-like activity of ceria nanozymes by crystal facet engineering. Reprinted with permission from ref. [27]; copyright (2023) American Chemical Society.

### 2.4. Doping

The specificity of nanozymes can be achieved by doping, as different elements exhibit varying binding strengths to substrates. For instance, the heteroatom doping strategy was employed to improve the peroxidase-like activity of reduced graphene oxide (rGO). Nitrogen (N) doping was found to significantly improve the peroxidase-like activity of rGO, while having negligible effects on the oxidase-, superoxide dismutase (SOD)-, and CAT-like activities of rGO (Figure 5A). To understand the mechanisms underlying this interesting phenomenon, density functional theory (DFT) calculations were performed. As summarized in Figure 5B, N-doped rGO (*i.e.*, N-rGO) selectively activated hydrogen peroxide but not oxygen or superoxide radicals by forming and stabilizing radical oxygen species adjacent to the N sites of N-rGO. The reactive oxygen species subsequently oxidized peroxidase substrates, achieving peroxidase-like catalysis. The N-doping strategy was also used to specifically enhance the peroxidase-like activities of other carbon nanozymes.[28-31] Additionally, metal-element doping has subsequently been demonstrated to adjust the catalytic selectivity of nanozymes. For instance, compared with $CeO_2$ nanozymes, Co-doped $CeO_2$ exhibited a higher CAT-like catalytic activity, while the Mn-doped $CeO_2$ tended to show SOD-like capacity.

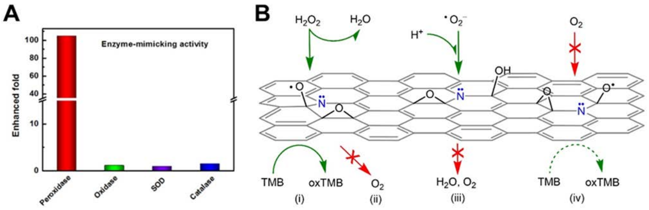


**Figure 5** Nitrogen doping specifically enhanced the peroxidase-like activity of carbon nanozymes. (A) Enhancement of the enzyme-like activities after N doping. (B) Mechanisms obtained by DFT calculations responsible for the specific peroxidase-like activity (i) but negligible CAT-, SOD-, and oxidase-like activities (ii, iii, and iv, respectively) of N-rGO. Reprinted with permission from ref. [28]; copyright (2018) American Chemical Society.

### 2.5. Molecularly imprinted polymers

Molecular imprinted polymers (MIPs) provide an effective strategy to endow a nanozyme with substrate specificity. In most cases, an entrapment method is used to *in situ* grow MIPs onto nanozymes. As shown in Figure 6, MIPs were grown onto peroxidase-like iron oxide nanozymes, resulting in a nearly 100-fold enhancement in substrate specificity.[32] Because MIPs are cost-effective and robust, and have a wide substrate adaptive capacity, other MIP-coated nanozymes have been developed for specific biorecognition.[33-36] Strictly speaking, MIPs do not endow nanozymes with intrinsic specificity. However, when considering MIP-coated nanozymes as integrative systems, they can be regarded as nanozymes with quasi-specificity.

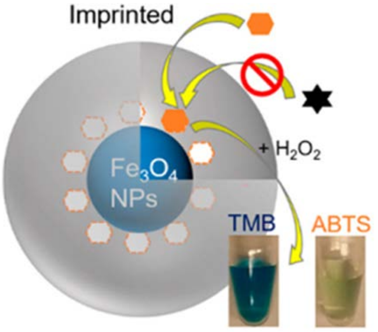


**Figure 6** Molecularly imprinted polymers. Reprinted with permission from ref. [32]; copyright (2017) American Chemical Society.

## 3. Strategies to Confer Analysis Specificity to Nanozymes in Applications

While nanozymes with intrinsic specificity have been exploited for analysis, such as specific peroxidase-like nanozymes for total antioxidant capacity assay,[29] a significant number of current nanozymes lack intrinsic specificity. To tackle this limitation, numerous strategies have been developed to combine the specificity of biological recognition elements (such as enzymes and antibodies) and other recognition techniques with the catalytic activity of nanozymes. These strategies are discussed below.

### 3.1. Combination with biorecognition elements

Most early studies focused on peroxidase-like nanozymes, which catalyze the oxidation of an organic substrate using hydrogen peroxide. Therefore, by combining with a hydrogen peroxide-producing enzyme (*e.g.*, an oxidase), the system could be used to selectively detect the substrate of the oxidase (Figure 7). For example, the combination of glucose oxidase, known for its specificity towards glucose, with a peroxidase-like iron oxide nanozyme enabled the selective detection of glucose.[9] Subsequently, other peroxidase-like nanozymes were also explored for glucose detection.[37-38] Other important targets, such as lactate and sarcosine, were successfully detected by combining the corresponding oxidase with a peroxidase-like nanozyme.[37,39] Moreover, by confining an oxidase and a peroxidase-like nanozyme together, integrated nanozymes could be obtained, which exhibited better cascade reaction efficiency and therefore led to more sensitive detection.[40-41]

An oxidase can be combined with other enzyme-like nanozymes for bioanalysis. For example, an alcohol oxidase was combined with a CAT-like Pd@Pt nanozyme for a drunken driving test.[42]

In addition to the combination with enzymes, the specificity of a nanozyme can be modulated by peptide (or nucleic acid) due to a couple of properties for bioanalysis. First, a peptide or nucleic acid may block the active sites of a nanozyme and decrease the activity. Second, the presence of a target molecule capable of binding to the peptide or nucleic acid can free the blocked active sites and recover the nanozyme activity. By taking advantage of these properties, numerous assays have been developed.[43-46] For instance, an AG-73 peptide can bind to a two-dimensional MOF (2D-MOF) nanozyme and inhibit its peroxidase-like activity. Through the specific interaction between heparin and AG73 peptide, the presence of heparin recovered the peroxidase-like activity, enabling selective and sensitive monitoring of heparin in blood.[44]

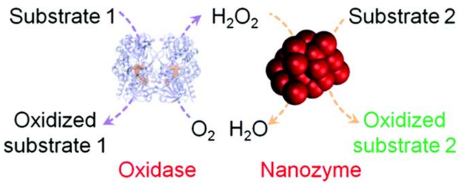


**Figure 7** Combination with an enzyme.

### 3.2. Surface modification with biorecognition elements

A biorecognition element can be conjugated onto a nanozyme to provide specificity for biomedical applications. Horseradish peroxidase (HRP)-antibody conjugate-based enzyme-linked immunosorbent assay (ELISA) is widely used. By replacing HRP with a peroxidase-like nanozyme, a similar ELISA can be developed. For example, by encapsulating peroxidase-like Pd–Ir nanozymes within an antibody-conjugated vesicle, a specific probe was developed. This probe was then used to form a sandwich ELISA for the detection of human prostate surface antigen (PSA), achieving a $10^3$-fold increase in sensitivity compared to conventional enzyme-based assays (Figure 8).[47] Other formats of ELISA, including later flow assays, have also been developed using antibody-modified nanozymes.[3,48-50]

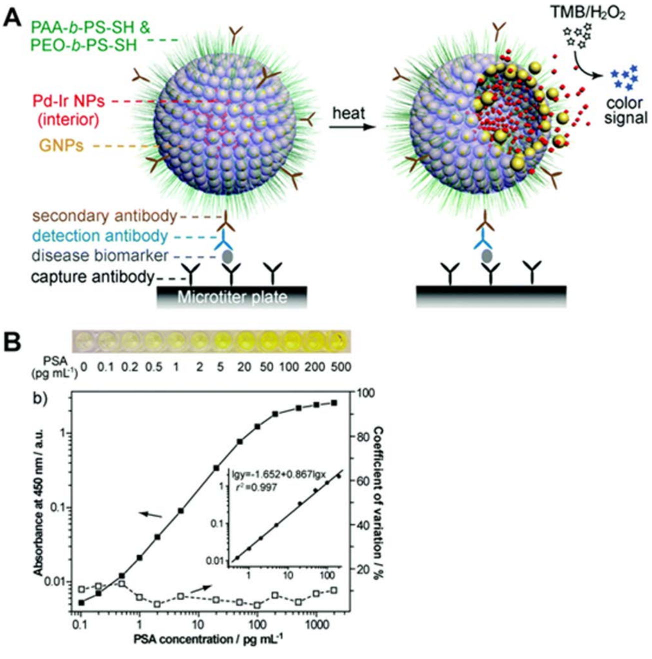


**Figure 8** Antibody-modified nanozymes as specific ELISA probes. (A) ELISA for ultrasensitive detection of disease biomarkers by using gold vesicle encapsulated Pd–Ir nanozymes for signal amplification. (B) Gold vesicle encapsulated Pd–Ir nanozyme-based ELISA for detection of PSA. Reprinted with permission from ref. [47]; copyright (2017) American Chemical Society.

Similarly, other biorecognition elements, such as aptamers and DNAzymes, can be conjugated onto nanozymes to create specific probes for biomedical applications.[51-52] Interestingly, recombinant human heavy-chain ferritin can bind to tumor cells that overexpress transferrin receptor 1. When ferritin was used to encapsulate iron oxide nanozymes, a specific staining probe was developed. This probe was used to examine clinical specimens from patients and demonstrated the ability to distinguish cancerous tissues from normal tissues with a sensitivity of 98% and specificity of 95%.[10]

### 3.3. Nanozyme sensor arrays

While the above-mentioned strategies are useful to address the specificity concerns of nanozymes, the use of biorecognition elements may compromise the high stability and low-cost promise of nanozymes in applications. It is known that mammalian gustatory and olfactory systems discriminate tastes and odors in a pattern-recognition fashion. Inspired by these systems, cross-reactive nanozyme sensor arrays have been developed to generate unique fingerprint-like patterns for multiplex detection.[53-57] As shown in Figure 9, peroxidase-like Pt, Ru, and Ir nanozymes were employed to develop a nanozyme sensor array.[53] These nanozymes exhibited varying degree of catalytic activity in the oxidation of *o*-phenylenediamine (OPD) in the presence of hydrogen peroxide. Leveraging the differential nonspecific interactions between the nanozymes and the analytes, biothiols, proteins, and cancerous cells were successfully identified. The identification of blind unknown samples was also achieved. Notably, discrimination of biothiols in serum and proteins in urine was also demonstrated.

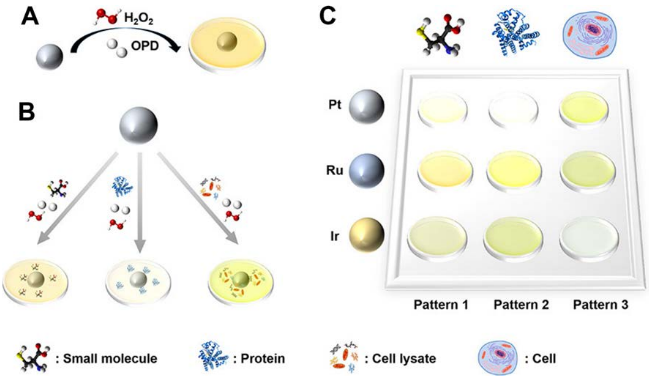


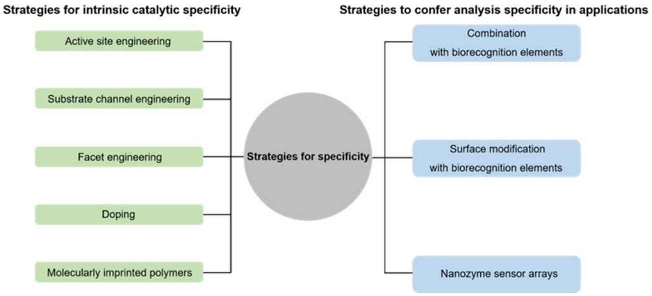
**Figure 9** Nanozyme sensor arrays for detecting versatile analytes. (A) Catalytic oxidation of OPD in the presence of peroxidase-like nanozymes. (B) Catalytic oxidation of OPD in the presence of nanozymes with small molecules, proteins, and cell lysates. (C) Pattern-based recognition of small molecules, proteins, and cells by Pt, Ru, and Ir nanozyme-based cross-reactive sensor arrays. Reprinted with permission from ref. [53]; copyright (2018) American Chemical Society.

Nanozyme sensor arrays can also be utilized to monitor enzymatic reactions.[54] For example, apyrase- and pyrophosphatase-catalyzed hydrolysis were tracked by the use of 2D-MOF-nanozyme-based sensor arrays.

## 4. Conclusions and Perspectives

Substrate specificity is critical when designing high-performance nanozymes. However, many currently developed nanozymes lack this specificity. To address this challenge, we have summarized eight promising strategies for modulating the specificity and selectivity of nanozymes (Scheme 1). Enzymes typically possess intricate and sophisticated structures, including substrate channels and active sites. To modulate the intrinsic specificity of nanozymes, it is possible to enhance the catalytic specificity by replicating these substrate channels, facet engineering/doping of nanozymes; on the other hand, utilizing MIPs to coat nanozymes is also an effective strategy to endow substrate specificity. In addition, several stopgap strategies have been designed to improve the specificity of nanozymes for analysis in applications. These strategies are straightforward, such as the combination/surface modification with biorecognition elements and nanozyme sensor arrays.

**Scheme 1** Strategies for endowing nanozymes with specificity



While these strategies have demonstrated success, further efforts are needed to develop highly specific nanozymes. In this regard, we would like to suggest several strategies for future studies.

1. Biomimetic design of nanozymes

An ideal strategy for enhancing the catalytic specificity of nanozymes is to imitate enzymes by studying their structures and functions, and introducing more dynamic and flexible soft structures into the generally rigid and hard structures of inorganic nanozymes.

First, the successful mimicking of heme cofactors shall inspire us to imitate other cofactors. For example, iron-sulfur clusters deserve more attention.[58]

Second, besides the cofactors, the microenvironment of active sites is of great importance. An active site usually has an optimal geometry to adapt to a substrate and/or the corresponding transient intermediate. Moreover, other physicochemical features should also be considered, including hydrogen-bonding networks, salt bridges, hydrophobic/hydrophilic interactions, *etc*.

Third, the delicate features of substrate channels should be more precisely imitated. For example, flexible molecular modification to the channels of inorganic nanozymes can enhance their functionality and affinity for substrates. Additionally, tailoring the hydrophobic or hydrophilic properties of the channels would provide optimized channel selectivity for target substrates. Utilizing the electrostatic attraction of opposite charges is another effective way to strengthen the interaction between substrates and nanozyme channels. For instance, if a substrate is positively charged, designing negatively charged channels can enhance the binding.

2. Computation-guided design of high-performance nanozymes

Current density functional theory (DFT) calculations focus on small molecules (*e.g.*, hydrogen peroxide, superoxide anion, and hydroxyl radicals) but are limited in predicting interactions with larger substrates like tetramethylbenzidine (TMB) and 2,2’-azino-bis-(3-ethylbenzthiazoline-6-sulphonate) (ABTS).[59] Integrating molecular dynamics (MD) simulations with DFT could offer more accurate predictions of interactions, thereby elucidating the catalytic mechanisms and enabling the design of nanozymes with specificity.

3. Prediction and design of nanozymes using machine learning

Machine learning (ML), a key facet of artificial intelligence, is adept at analyzing extensive datasets, offering promising avenues for the specificity and gradual improvements of nanozymes. By integrating experimental data with ML-driven data mining and performance prediction, this approach plays a crucial role in unraveling the intricate structure-function correlations in nanozymes. Notably, the application in ML field is emergent, primarily drawing upon data from specialized nanozyme databases.[60-61] Therefore, a pivotal focus should be on the accumulation and enrichment of experimental datasets. The utilization of quantitative comparisons ($k_{cat}/K_M$ or $k_{cat}$) to assess nanozyme specificity enhances the precision in contrasting various reactions or substrates, thereby laying a robust groundwork for ML’s implementation in nanozyme innovation.

4. Unlike enzymes, it is worth noting that the specificity of nanozymes could also be improved through structural engineering and external stimuli.[62-64] Additionally, it is important to recognize that not all enzymes exhibit strict specificity. Some enzymes have multiple specificity while others exhibit promiscuity.[65] This introduces a dialectical relationship regarding specificity and multienzyme activity between enzymes and nanozymes.

Overall, by learning from enzymes and exploring the unique properties of nanomaterials, highly specific nanozymes can be designed.[1-2,8,23,66]

## Acknowledgement

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