nature reviews methods primers

https://doi.org/10.1038/s43586-024-00315-5

Primer

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Nanozymes for nanohealthcare

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Abstract

Nanozymes, nanomaterial-based artificial enzymes, exhibit potential for emulating the catalytic functions inherent in enzymes. Nanozymes have advantages such as low cost, facile synthesis, high stability and adjustable activities. As a promising approach for healthcare, nanozymes have sparked considerable interest, and have been chosen as one of the 2022 Top Ten Emerging Technologies in Chemistry by the International Union of Pure and Applied Chemistry (IUPAC). This Primer provides theranostic insights from the nanozyme toolbox, encompassing the design of nanoparticles, evaluation of activities and applications. We focus on rational strategies to enhance nanozyme activities, emphasizing standardized evaluations across different activities, and outline specific details for their practical applications. The selection of candidates for diagnosis, as well as those for in vivo theranostic applications, is carefully considered based on appropriateness. We also acknowledge current challenges and limitations, presenting future perspectives and positioning nanozymes as an alternative and effective choice in theranostics. This Primer aims to contribute to the understanding and advancement of nanozyme applications in healthcare, offering a comprehensive guide for researchers in this dynamic field.

Sections

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Introduction

Enzymes have wide applications in biomedicine, owing to their catalytic ability. Enzymatic interventions to address genetic deficiencies are focused on substances involved in metabolism that serve as substrates or products in designed catalytic reactions. Although current therapeutic enzymes have shown promise in treating diseases[1], they are specialized and often need to be used in combination. Moreover, enzymes face inherent limitations in practical applications, such as easy inactivation and the need for effective delivery systems[2].

Nanomedicine involves the use of nanotechnology for diagnostics and therapeutics[3]. To date, liposomes[4], iron oxide nanoparticles[5] and gold nanoparticles[6], among others, have been developed as nanocarriers, magnetic resonance imaging reagents and photothermal therapy (PTT) reagents, owing to their amphiphilic, magnetic and optical properties. Nevertheless, the catalytic ability inherent in nanomaterials was largely overlooked in the early development of nanomedicine[7–11].

Nanozymes are functional nanomaterials with enzyme-like activities[12–15]. The discovery of intrinsic peroxidase-like activity of Fe_3O_4 nanoparticles sets off a trend in nanozyme research[16]. Compared with their natural counterparts, nanozymes exhibit advantages such as economic affordability, robust stability and diverse physico-chemical properties. These advantages not only effectively address the instability and cost challenges associated with enzymes but also present potential for rational design and functionalization[12–14]. Meanwhile, nanozymes exploit the inherent characteristics of nanomaterials, including mechanics[17], magnetism[18], optics[19] and electricity[20]. These attributes have found applications in nanozyme-related diagnosis and therapy. Compared with enzymes, an intriguing aspect of nanozymes is their ability to achieve self-cascade reactions owing to their multi-enzyme-like activities. This feature facilitates an integrated approach that reduces mass transfer limitations across spatio-temporal distances[21]. The potential for self-cascade reactions broadens the scope of applications for nanozymes in various therapeutic and diagnostic scenarios, with promising advancements in nanomedicine[22,23].

Nanozymes have found increasingly wide utilization in healthcare, similar to enzyme replacement therapy, which is based on catalytic reactions in metabolism. In an ideal process, after confirming the target in diseases, a designed nanozyme can be used as a diagnostic agent or a therapeutic drug. Besides evidence-based synthesis (Box 1), the rational design of nanozymes through structure–activity relationships is gradually becoming a consensus[24]. Refined manipulation at the atomic scale and the nanoscale can result in a high-performance nanozyme for a given reaction and, in turn, the regulation strategy can guide the design of other nanozymes. Following the acquisition of requisite nanozymes, surface engineering becomes essential to impart non-catalytic properties, such as specificity to target molecules or targeting diseased sites of the nanomedicine itself. The modified nanozyme can serve as a modulator to alter surface acidity and charge, and even influence the adsorption and desorption of intermediates[25] (Fig. 1). The primary structure of nanozymes can be established from the initial design, whereas the modification of nanozymes can be regarded as the secondary structure. The modified nanozymes can be used for biomedical applications including diagnostics and therapeutics. Increased activity generally lowers the limit of detection and enhances the outcome of therapy, whereas enhanced selectivity prevents interference from other substances, establishing a robust basis for analytical and therapeutic applications.

Once synthesis has been optimized, physico-chemical and enzymatic properties of nanozymes should be determined for the intended application (Fig. 1). Initial characterization methods to confirm the structure of nanozymes should include determining the size, shape, surface condition and dispersion to ensure they are suitable for the intended application. For example, the kidneys' filtered threshold is 5.5 nm. Nanozymes smaller than this threshold can be excreted, indicating potential for trans-urinary metabolic theranostics[26]. Furthermore, the assessment of enzymatic activities is crucial for verifying the catalytic capabilities of nanozymes[27]. Numerous enzyme-like activities, such as superoxide dismutase, peroxidase, catalase, oxidase and hydrolase, have found biomedical applications[28]. Various approaches for assaying enzymic activities have been explored[27,29–32]. Most commonly, changes in the absorbance or fluorescence of reacting substrates or products serve as indicators for activity of nanozymes.

For nanozymes as diagnostic agents, interference studies should then be conducted to assess the stability, specificity and sensitivity of nanozymes under working conditions. For in vivo diagnostics, biocompatibility and metabolism should be considered. During pilot studies, it is important to screen an analytical method and administration

Box 1

Common methods for nanozyme synthesis

Various methods can be employed to produce the desired nanozymes, including hydrothermal, co-precipitation, sol–gel, pyrolysis and microwave radiation methods. Each method offers unique advantages and is suitable for different types of nanozymes and their applications[193]. For instance, a hydrothermal method involves the use of high-temperature and high-pressure conditions in an aqueous solution to promote the synthesis of nanostructures[194]. It is often favoured for its simplicity and ability to produce highly crystalline nanozymes. The components and structures of nanozymes can be modulated by varying conditions such as reaction time, temperature and precursor concentration. In a co-precipitation method, two or more precursor salts are dissolved in a solvent, and a precipitant is added to induce the formation of insoluble nanoparticles. This is a straightforward technique for producing homogeneous nanozymes such as Fe_3O_4 nanoparticles[117]. The sol–gel method involves the formation of a sol (a stable colloidal suspension of nanoparticles) followed by gelation to form a solid gel. It is known for its versatility in producing various shapes and sizes of nanozymes with controlled porosity[49]. Pyrolysis involves the decomposition of organic precursors at high temperatures in an inert atmosphere to form nanostructured materials. It is particularly useful for synthesizing carbon-based nanozymes with unique properties (for example, carbon-based single-atom nanozymes[89]). A microwave irradiation method utilizes microwave radiation to heat reaction mixtures rapidly and uniformly, leading to accelerated synthesis processes compared with conventional heating methods. The microwave irradiation method has been utilized to synthesize a range of nanozymes including carbides, nitrides, sulfides, oxides and metal–organic frameworks (MOFs)[193,195].

mode that aligns with the disease of interest. For nanohealthcare-related analysis, various methods have been employed, including the nanozyme-linked immunosorbent assay[33], test strips[34,35] and sensor assays[36]. Notably, the nanozyme-assisted strip gained recognition for its rapid local diagnosis of Ebola[34]. In therapeutic applications, the choice of administration method and the nanozyme dosage are of significance. A low yet efficacious dose is desired, considering factors ranging from cost to biosafety. As the formal assessment commences, the insights from the pilot results are important for optimizing the treatment plan. For example, if outcomes related to biosafety indicators, such as pharmacokinetics, prove suboptimal, adjustments to the nanozyme dosage should be made. This iterative process ensures that the therapy remains both effective and safe.

There are two types of nanozyme-assisted therapy; the first is based on catalysing the conversion of substrates that are often overexpressed in diseased sites, and the other relies on producing products that are helpful to treat diseases. For example, in conditions characterized by an imbalance in reactive oxygen species (ROS), such as organ injury[37], depression[38] and orthopaedic disease[22,39], nanozymes with catalase-like and superoxide dismutase-like activities can effectively scavenge excessive ROS. This approach aims to restore redox balance by reducing the elevated levels of ROS. By contrast, when dealing with cancer, the goal is to induce the production of ROS to selectively target and eliminate malignant tumour cells. Nanozymes facilitate this process through targeted and controlled generation of ROS for therapeutic purposes.

This Primer aims to give an overview of nanozyme preparation, from nanomaterial design to activity evaluation and theranostic applications, and a guide to analyse and validate the efficacy of nanozymes. Furthermore, concrete steps to enhance experiment reproducibility and the reliability of data deposition are discussed. Finally, challenges and limitations in the field for presenting perspectives of nanozyme-assisted healthcare are presented. Nanozymes are considered an alternative, effective and promising choice in the ongoing evolution of therapeutic strategies. This Primer seeks to contribute to the knowledge in nanomedicine, especially fostering a deeper understanding of nanozyme applications in healthcare.

Experimentation

Nanozymes present an avenue to build a platform for precision medicine[40,41]. Precision medicine aims to design specific therapeutic and diagnostic strategies based on disease indicators, demanding both high sensitivity and specificity from the chosen interventions. In this section, the key factors involved in nanozyme design, surface engineering and the experimental flow of nanozyme application are discussed.

Design of nanozymes

Trial-and-error method. High-performance nanozymes are often obtained using a trial-and-error approach[12] (Fig. 2a). In this approach, different materials with diverse structures are synthesized under various conditions to find the optimal conditions through iterative testing. The size of nanoparticles is a pivotal factor influencing catalytic activities; smaller nanoparticles typically exhibit increased activities, attributed to their larger surface area, which increases the number of active sites[42]. The composition and facets of nanoparticles are also crucial for catalytic activities. Generally, noble metals, such as platinum, are often regarded as good oxidase mimics whereas nanozymes composed of variable valent metals, such as cerium and manganese, may have good superoxide dismutase-like activity. These nanozymes

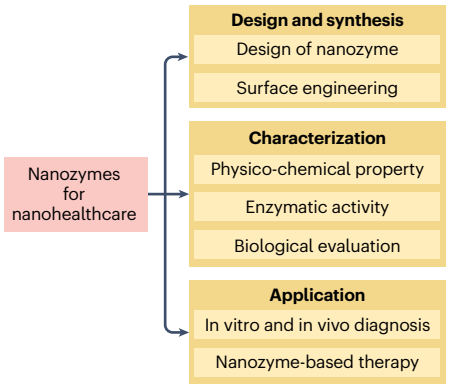

Fig. 1 | Concept of nanozymes for nanohealthcare. Workflow of nanozymes for nanohealthcare involves design and synthesis, characterization and application.

demonstrate varying levels of activity when they expose different crystal facets. Transmission electron microscopy (TEM) or X-ray powder diffraction (XRD) can be used to identify facets and composition associated with enhanced performance. Employing suitable synthesis methods allows for the deliberate exposure of specific facets, further optimizing catalytic activity[43,44].

Structure–activity relationship-directed design of nanozymes. Structure–activity relationship-directed design has emerged as a powerful approach for material design. This method involves constructing materials with a deep understanding of their physico-chemical properties, including interfaces. By fine-tuning these materials based on experimental data, researchers can identify performance indicators, often described as activity descriptors[45] (Fig. 2b).

To understand activity descriptors, it is essential to first understand catalytic reactions. The adsorption or desorption of reactants and products determines the efficiency of the catalytic reaction; understanding and manipulating these interactions can aid the design of nanozymes with excellent catalytic activity. Intermediates are transient species that exist between the reactants and products, which should also be considered during the catalytic process. Intermediates often represent key points in the reaction pathway and can influence the overall efficiency and selectivity of the catalytic process. Through computation, these intricate processes can be simulated and analysed, allowing for a more systematic approach to nanozyme design. These principles are embodied in descriptors.

Nanozyme-catalysed reactions involve critical surface intermediates that play a pivotal role in enzyme-like activities. The understanding of these surface processes has been guided by principles in heterogeneous catalysis. The Sabatier principle has provided a valuable framework for correlating various factors, such as adsorption energy and activity[46]. This principle suggests that there exists an optimum interaction strength between a catalyst and its reactants or intermediates for achieving the highest catalytic activity. For example, the energy of the *d* states serves as a classic descriptor for evaluating catalyst–substrate interaction[47,48]. The *d*-band theory provides insights into the catalytic activity of metals based on their electronic structures, namely, a higher energy position of the metal's *d* states relative to the Fermi level signifies less availability of antibonding states. This, in turn, corresponds to stronger adsorption in catalytic processes.

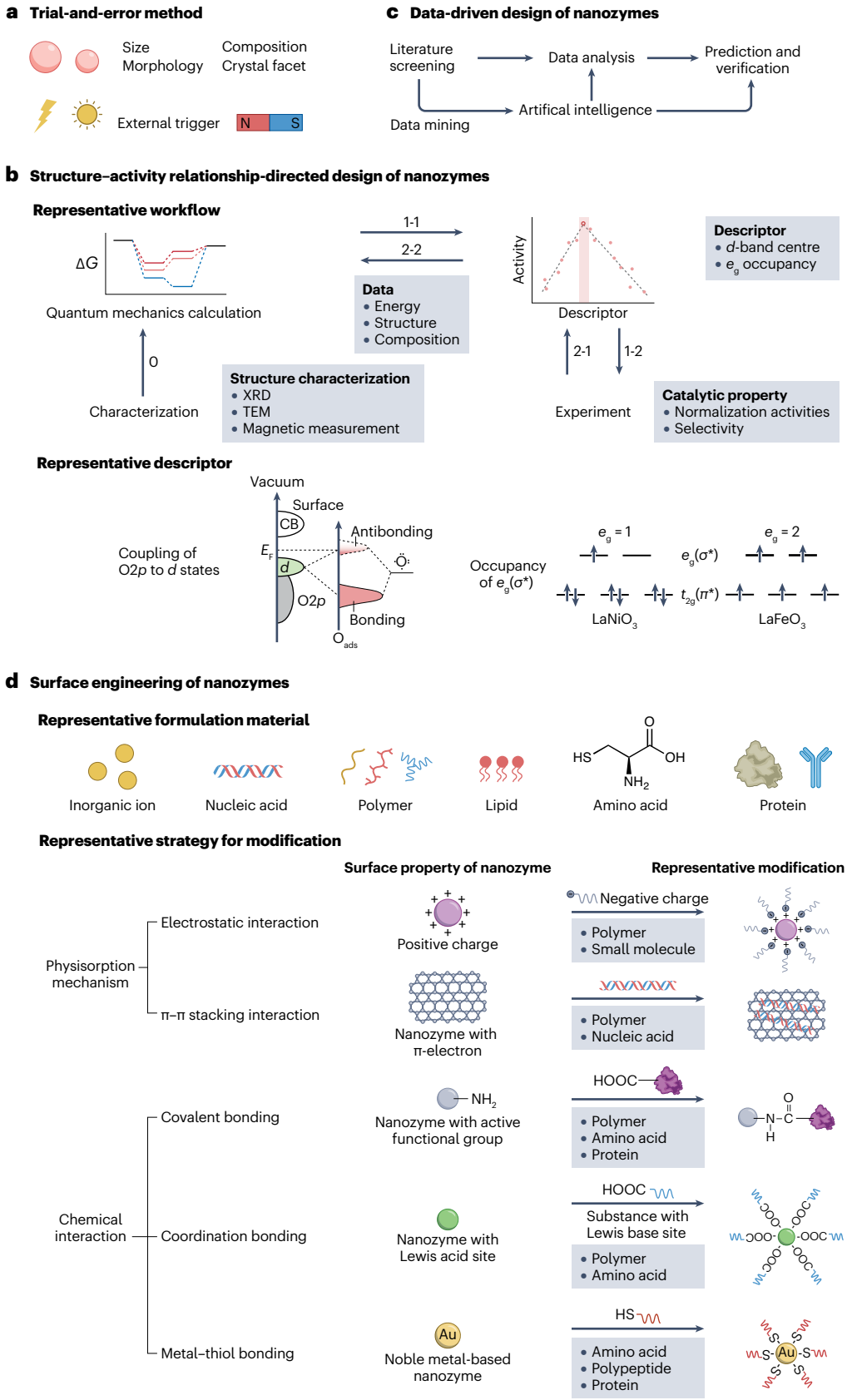
a Trial-and-error method
Size
Morphology
Composition
Crystal facet
External trigger
N
S
c Data-driven design of nanozymes
Literature screening
Data analysis
Prediction and verification
Data mining
Artifical intelligence
b Structure–activity relationship-directed design of nanozymes
Representative workflow
ΔG
Quantum mechanics calculation
0
Characterization
1-1
2-2
Data
• Energy
• Structure
• Composition
Structure characterization
• XRD
• TEM
• Magnetic measurement
Activity
Descriptor
2-1
1-2
Experiment
Descriptor
• d-band centre
• e_g occupancy
Catalytic property
• Normalization activities
• Selectivity
Representative descriptor
Coupling of O2p to d states
Vacuum
Surface
CB
E_F
d
O2p
Antibonding
Bonding
O_ads
Occupancy of e_g(σ*)
e_g = 1
e_g = 2
e_g(σ*)
t_2g(π*)
LaNiO_3
LaFeO_3
d Surface engineering of nanozymes
Representative formulation material
Inorganic ion
Nucleic acid
Polymer
Lipid
Amino acid
Protein
Representative strategy for modification
Surface property of nanozyme
Representative modification
Physisorption mechanism
Electrostatic interaction
π–π stacking interaction
Chemical interaction
Covalent bonding
Coordination bonding
Metal–thiol bonding
Positive charge
Nanozyme with π-electron
Nanozyme with active functional group
Nanozyme with Lewis acid site
Noble metal-based nanozyme
Negative charge
• Polymer
• Small molecule
• Polymer
• Nucleic acid
HOOC
• Polymer
• Amino acid
• Protein
HOOC
Substance with Lewis base site
• Polymer
• Amino acid
HS
• Amino acid
• Polypeptide
• Protein

Fig. 2 | Rational design and surface engineering of nanozymes. a, Employing the trial-and-error method, nanozyme design focuses on crucial factors such as size, morphology, composition, crystal facet and external triggers. **b**, Structure–activity relationship-directed design of nanozymes. Material characterization provides information on composition, structure and energy, which can be input into calculations to establish models for further processes (step 0). Calculations yield a set of descriptors, some of which are predicted to be related to nanozyme activities (step 1-1). Subsequently, nanozymes exhibiting the best-predicted activities are synthesized, and their activities and selectivity are assessed (step 1-2). Alternatively, researchers synthesize a range of nanozymes and evaluate their activities. They then deduce a descriptor that illustrates the structure–activity relationship based on test data (step 2-1). To validate this relationship, quantum mechanics calculations are employed for thorough verification (step 2-2). **c**, In data-driven nanozyme design, the process begins with literature screening. Artificial intelligence facilitates this by transforming manual literature screening into efficient data mining. Following human or artificial intelligence-supported summary and analysis, predictions are made and verified by experiments. **d**, Representative formulation materials for surface engineering of nanozymes: inorganic ion, amino acid, polymer, lipid, nucleic acid and protein such as antibody (bovine serum albumin (PDB:4F5S)). Representative strategy for surface engineering: physisorption mechanism (for example, electrostatic and π–π stacking interaction) and chemical interaction (covalent, coordination and metal–thiol bonding). CB, conduction band; TEM, transmission electron microscopy; XRD, X-ray powder diffraction.

Similar to the *d*-band centre, the e_g electron can also be regarded as a descriptor. This descriptor has been investigated in perovskite or spinel transition metal oxides (TMOs) and their peroxidase-like activity[49,50]. The splitting of the *d* orbital of transition metal sites in an octahedral crystal would form two antibonding molecular orbitals (such as $\sigma^*_{x^2-y^2}$ and $\sigma^*_{z^2}$), which are referred to as the e_g orbitals. e_g occupancy represents the *d*-electron population of the e_g orbitals. The occupancy of these orbitals has a governing role in influencing the peroxidase-like activity of perovskite or spinel TMOs. By exploring the e_g occupancy, the relationship between electronic structure and catalytic behaviour of TMOs can be better understood. This understanding guides the design of TMOs with optimal peroxidase-like activity, paving the way for developing efficient and versatile nanozymes.

Computed results, obtained through computational modelling and simulations, such as density functional theory calculations, are important in understanding the reaction processes of nanozymes[51,52]. Density functional theory calculations provide a versatile platform for exploring the thermodynamics and kinetics inherent in nanozyme-catalysed processes. The principles derived from density functional theory calculations can effectively forecast the activities of nanozymes, including superoxide dismutase-like activity, which offers valuable insights into their catalytic behaviour and screening approach for given enzyme-like activity[52]. These results can help understand the electronic structure, surface properties and energetics of nanozymes. By combining with experimental results, a set of descriptors can be established to predict activities to uncover the underlying mechanisms that govern the behaviour of molecules and their role in chemical reactions and biological processes.

As the field of nanozymes continues to advance, the descriptors used to understand and predict their catalytic activities must also evolve. For nanozymes with different catalytic activities, descriptors should be tailored to suit the specific reaction mechanisms and behaviours of each nanozyme. The other key to reliable and effective nanozyme design lies in making meaningful connections between theoretical predictions and experimental outcomes. By confirming and fine-tuning models based on experimental data, researchers can develop dependable strategies for the rational design of nanozymes with different enzyme-like activities.

Data-driven design of nanozymes. There is a rich repository of invaluable data, offering insights into previously undiscovered aspects of nanozyme behaviour. A strategic approach involves the collection and systematic analysis of research papers relevant to the research question to determine patterns and trends, which can serve as guiding principles to facilitate exploration to inspire innovative ideas for nanozyme design (Fig. 2c).

A series of hydrolytic nanozymes have been discovered following a data-driven strategy[53], such as metal–organic frameworks (MOFs); MOF-based hydrolase mimics show higher representation and better performance in the literature than other materials. Furthermore, Lewis acid-based metal clusters exhibit pronounced affinity towards hydrolysis substrates. Through finely tuning the size of organic linkers, a specifically designed nanozyme can be synthesized, showcasing remarkable performance, particularly for phosphatase-like activity. Successful implementation of this approach requires careful analysis of the gathered data, coupled with the discerning utilization of intuition to generate a novel design tactic.

Data acquirement (or data mining) is essential to data-driven design of nanozymes. Data mining represents a comprehensive process encompassing the extraction, organization, analysis, summarization and integration of vast data sets[54]. The primary objective is to unearth valuable information residing in the expansive layers of data, such as discerning trends, identifying features, recognizing patterns and unveiling hidden relationships. Within artificial intelligence, machine learning emerges as a distinct and highly accessible data mining method. Over recent years, machine learning has experienced significant development. Machine learning, within the data mining framework, automates the analysis process by adeptly extracting patterns from the data, subsequently employing these patterns to make predictions regarding unknown data. This automated learning capacity is implemented through algorithms meticulously designed for predicting specific characteristics. Through experimentation verification, artificial intelligence can be utilized to enhance the performance of nanozymes through iterative feedback loops. Increasingly, this artificial intelligence–human collaboration-based design principle is playing a crucial role in the rational design of nanozyme activity[55].

Surface engineering of nanozymes

Surface engineering of nanozymes is an effective strategy to modulate their activity, selectivity and targeting ability[56–58]. Surface engineering of nanozymes is quite straightforward and robust, and the surface chemistry of the nanozymes dictates the strategy used. For example, negatively or positively charged nanozymes can adsorb molecules with opposite charges on their surface. This can be achieved through incubation of pristine nanozymes within solutions of ions or molecules. To remove free ions or molecules, centrifugation can be employed to wash the modified nanozymes. If the nanozymes are too small for centrifugation, dialysis can be used, whereas magnetic separation is ideal for magnetic nanozymes. Surface engineering of nanozymes includes strategies such as electrostatic interaction, π–π stacking interaction, covalent bonding, coordination bonding and metal–thiol bonding.

Nanozymes possess π-electrons that can interact with the π-electrons on aromatic molecules. Functional groups on the surface of nanozymes can be employed to conjugate molecules covalently. In addition, metal species on the surface of nanozymes can have relatively strong interactions with Lewis base groups; if noble metals are present, nanozymes will react with thiol groups. These enable abundant molecules to modify nanozymes.

Surface engineering for activity modulation. Surface modification is effective for fine-tuning the catalytic activity of nanozymes[58]. A diverse array of inorganic ions[59], small molecules[60], polymers[61] and biomacromolecules[62] have been harnessed to modulate the catalytic behaviour of nanozymes (Fig. 2d). The modification of nanozymes with ions and molecules primarily involves physisorption mechanisms, such as electrostatic interactions[63]. In some cases, molecules can be covalently attached or coordinated[64] to the surface of nanozymes[65]. Typically, the active sites of nanozymes are located on their surface, and this is where modification can play a pivotal role. The modification can either obstruct these catalytic sites, leading to a reduction in activity, or create an environment to enhance the activity. Such enhancements are achieved by establishing a tailored chemical microenvironment that facilitates the local accumulation of substrates[66], improves substrate affinity[67], enhances product desorption[68] and promotes efficient charge transfer[69]. For instance, amino acids, with abundant amino and carboxyl groups, can be utilized to modify peroxidase-like Fe_3O_4 nanozymes, resulting in a boost in their catalytic activity[70]. In addition to improved affinity for H_2O_2, modification with negatively charged biomolecules such as DNA can attract positively charged substrates such as 3,3′,5,5′-tetramethylbenzidine (TMB) through electrostatic forces, leading to an increase in activity. In another example, fluoride ions can be adsorbed onto the surface of cerium oxide (CeO_2), thereby enhancing the oxidase-like activity of CeO_2 through the facilitation of charge transfer processes[69]. In brief, modification serves as a versatile tool for optimizing the catalytic performance of nanozymes by either fine-tuning their active sites or creating a conducive chemical environment. Moreover, modification can also improve fine dispersity of nanozymes in reaction mediums.

Surface engineering for substrate selectivity modulation. In addition to activity modulation, various modification strategies can be employed to fine-tune the substrate selectivity of nanozymes. Electrostatic interactions serve as a potent strategy for tuning the substrate reactivity. Negatively charged nanozymes have enhanced activity towards positively charged substrates, but can repel negatively charged substrates, such as 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), leading to an improved substrate selectivity towards TMB[62]. To avoid the activity inhibition of nanozymes for certain substrates, electrostatic repulsion between the surface and substance should be noted. Steric effects represent another strategy for modulating substrate selectivity, particularly in enantioselective reactions where numerous chiral ligands have been utilized to modify nanozymes. For instance, D-cysteine-modified gold nanozymes exhibit a preference for catalysing the peroxidation of L-3,4-dihydroxyphenylalanine (L-DOPA), whereas L-cysteine-modified gold nanozymes tend to catalyse the peroxidation of D-DOPA[71]. Furthermore, additional modification strategies offer advantages in governing the substrate selectivity of nanozymes. One noteworthy example involves the coating of molecularly imprinted polymers, which can provide Fe_3O_4 nanozymes with a 100-fold increase in substrate selectivity[72]. Modification with aptamers, selected to bind certain targets, also improve substrate selectivity[65]. In general, when considering the selectivity towards chiral substrates, chiral ligands should be used to modify nanozymes. To achieve the selectivity towards a specific substrate, use of molecularly imprinted polymers or aptamers is effective.

Surface engineering for targeting. There are numerous modification strategies for targeted applications. Antibodies can be employed to customize the surface of nanozymes, enabling them to precisely target specific antigens in immunoassays[16]. Beyond antibodies, nanozymes can also be coated with other ligands, such as ferritin, to target tumour cells. Ferritin exhibits an affinity for the transferrin receptor 1, which is often overexpressed on the surface of tumour cells. The cavity of ferritin is a reaction chamber to load metal ions and then condensate for mineralization[73]. By encapsulating nanozymes within ferritin, it becomes possible to target tumour cells and visualize tumour tissues[74]. Furthermore, biomacromolecules can be used as a modification strategy to target macrophages. For instance, hyaluronic acid exhibits a specific binding affinity towards CD44 receptor, which is commonly overexpressed on the surface of macrophages. Functional groups such as the carboxyl groups in hyaluronic acid can serve as covalent conjugation sites for nanozymes. Nanozymes modified with hyaluronic acid effectively target macrophages at inflammatory sites, offering potential treatment for inflammation[75]. In an alternative strategy, nanozymes can be encapsulated within negatively charged polymers, such as alginate, facilitating their targeting abilities through electrostatic interactions. This strategy has been harnessed for the specific targeting of ulcerative colitis[76]. To boost the spatio-temporal precision and effectiveness of nanozymes, employing dual modification can pave the way for a cascade-targeting approach. For example, nanozymes modified with hyaluronic acid and (3-carboxypropyl)triphenylphosphonium bromide target the CD44 receptor on tumour cells and mitochondria sequentially[77]. These diverse modification strategies underscore the adaptability of nanozymes in targeting precise cellular or tissue sites, making them important for various applications.

Experimental flow of nanozyme applications

After designing and synthesizing nanozymes, a specific emphasis on their application is necessary. Nanozyme-based nanohealthcare includes both diagnosis and therapeutics. In this context, the experimental flow for both is discussed.

In vitro or in vivo diagnosis. In diagnostics, nanozymes are used to detect biomarkers, pathogens and abnormal cellular activities with high sensitivity and selectivity. Rational design endows the nanozymes with high performance. To achieve selectivity, nanozymes are often conjugated with recognition moieties, enabling them to specifically recognize targets[41] (Fig. 3a). Two main approaches to diagnosis using nanozymes are nanozyme-involved reactions and nanozymes as catalytic tags. In nanozyme-involved reactions, a cascade reaction transforms a substance that is challenging to detect into an easily detectable one. Enzymes, such as oxidase, which can catalyse the conversion of small molecules (for example, glucose, uric acid and cholesterol) to H_2O_2 are combined with nanozymes for the cascade reactions. H_2O_2, acting as a reactant for peroxidase-like nanozymes, oxidizes substrates such as TMB or Amplex Red, producing oxidized products with colorimetric or fluorescent signals. The signal intensity correlates with the concentration of the targets, providing insights into disease severity. The second approach, nanozymes as catalytic

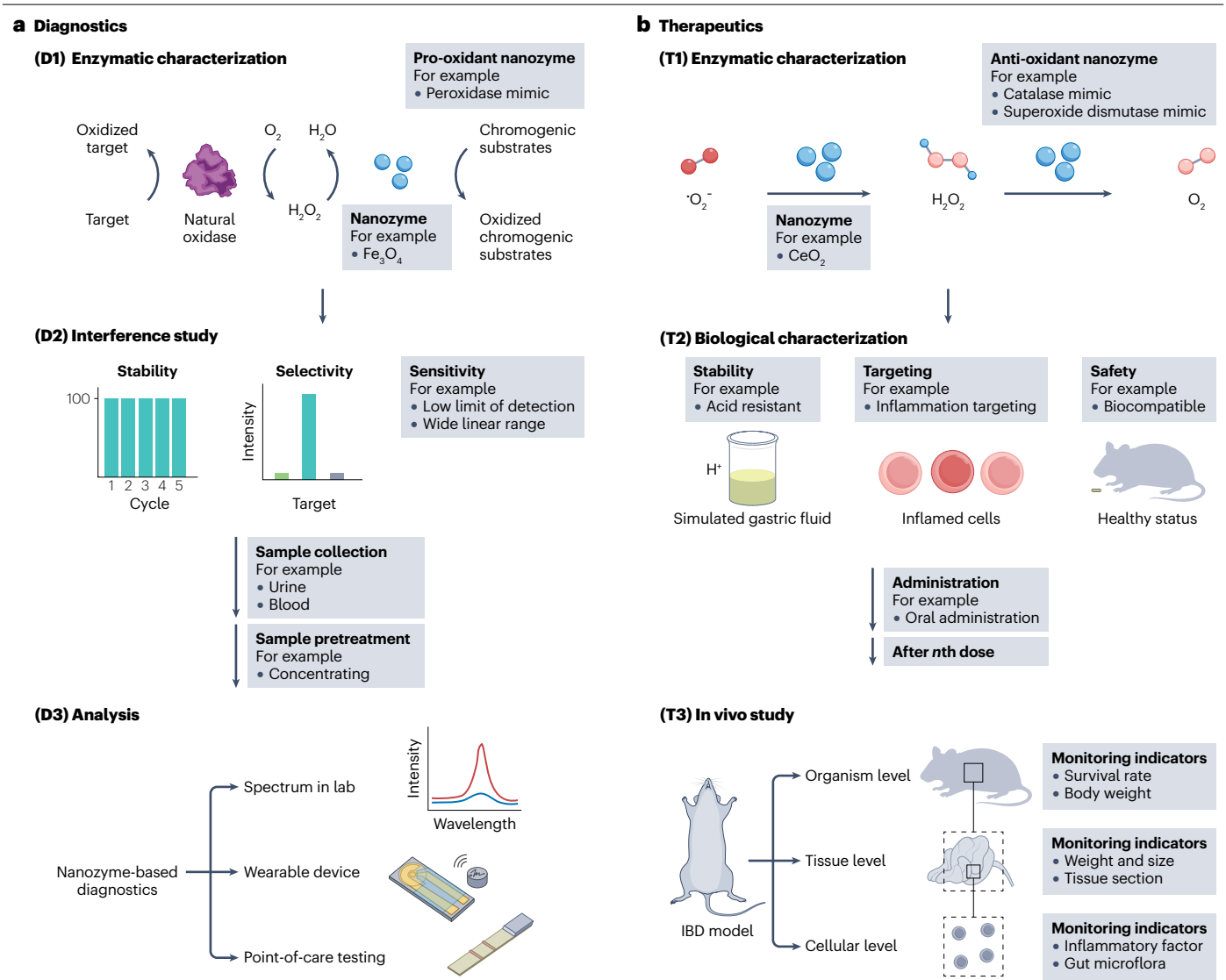

Fig. 3 | Experimental flow of nanozyme-based diagnostics and anti-oxidant nanozymes for inflammatory bowel disease. a, The typical procedure for nanozyme-based diagnostics comprises three steps. First, enzymatic characterization of pro-oxidant nanozymes is evaluated by chromogenic reaction (D1). An interference study should then be conducted, which involves stability, selectivity and sensitivity (D2). Next, samples are obtained from patients or animal models, through collection of representative body fluids such as sweat, tears, blood, urine and saliva. Following necessary treatment (such as concentrating), the targets undergo recognition and detection through catalytic reactions. Finally, the signals indicating the health status are demonstrated by absorbance and other visualizable approaches (D3). **b**, Experimental workflow for anti-oxidant nanozymes in the treatment of inflammatory bowel disease (IBD). Initially, the anti-oxidant capability of nanozymes is verified (T1). Subsequently, the nanozymes undergo evaluation for nano–bio interactions, such as acid resistance in simulated gastric fluid, inflammatory targeting ability and biocompatibility (T2). Following characterization, the nanozymes are administered through various methods, such as oral ingestion. After the administration of the *n*th dose, data are collected for analysis to validate the efficacy of the nanozymes at organism, tissue and cellular levels (T3). CeO_2, cerium oxide.

tags, involves modification on the surface of nanozymes to detect targets. Elements with recognition capabilities, such as antibodies, are employed to capture targets in blood, urine or other body fluids. These captured targets are then detected through catalytic reactions of nanozymes. Commonly used methods within these approaches include the nanozyme-linked immunosorbent assay, test strips and other assays. These techniques offer high accuracy and rapid detection, providing user-friendly and real-time monitoring capabilities. By leveraging the versatility of nanozymes, these approaches contribute significantly to advancements in healthcare, facilitating precise and efficient disease detection and monitoring[78].

In vivo therapies. In therapeutics, nanozymes are employed to accelerate reactions that facilitate the elimination of excess substances or that supplement reactions lacking substances. To effectively employ nanozymes for therapeutics, it is crucial to understand and target the

pathologic microenvironment of diseases. Biology developments provide insight into disease progression, revealing system dysfunctions at the molecular level, which aids exploration of nanozyme-based therapies. In diseased states, harmful substances tend to accumulate, owing to the dysfunction of enzymes or protective mechanisms. Nanozymes scavenge excessive harmful substances, mitigating damage caused by their accumulation. Additionally, they facilitate the release of beneficial substances that serve as crucial signal messengers. This process involves the required molecules from both endogenous and exogenous supplementation. Given the inherent and tunable catalytic abilities of nanozymes, the optimal activity of a therapeutic nanozyme should match the microenvironment at the diseased sites to fulfil its efficacy. Microenvironment characteristics such as pH, temperature and oxygen levels should be considered when designing therapeutic nanozymes. Additionally, it is important to account for potential poisoning of nanozyme active sites during the design process.

The administration routes, based on the specific requirements in diseases, affect the choice of nanozymes[79]. Common administration approaches include intraperitoneal injection, oral administration and intravenous administration. In certain medical scenarios, particularly in treating infections and certain cancers, therapeutic methods often gravitate towards in situ treatment, directly targeting the diseased areas. In animal studies, intraperitoneal injection stands out as a commonly employed method for delivering nanozymes, owing to the rich blood supply in the peritoneum. This route proves suitable for investigations involving systemic delivery or the targeted delivery of nanozymes to specific organs within the abdominal cavity. Oral administration represents a patient-friendly delivery approach. However, the harsh conditions within the stomach pose challenges for the delivery of non-acid-resistant nanozymes and intestinal peristalsis accelerates the excretion process. To overcome this hurdle, robust and targeted carriers can be employed to facilitate the effective delivery of nanozymes, particularly in the treatment of gastrointestinal diseases[76] (Fig. 3b). For deep tissue diseases or illnesses affecting the bloodstream, intravenous administration is a preferred choice. This method entails directly injecting nanomedicines into the bloodstream, ensuring rapid and widespread distribution throughout the body. This approach proves effective in addressing conditions that require a systemic impact or involve deep-seated tissues. In all these approaches, the key considerations revolve around pharmacokinetics, which encompasses distribution, metabolism and elimination. Understanding how nanozymes move within the body, undergo metabolic processes and are, eventually, eliminated is crucial for optimizing their therapeutic efficacy (Fig. 3b).

Beyond traditional 'drug-like' treatments, significant efforts have been dedicated to the development of implants or devices incorporating nanozymes[39,80,81]. These engineered implants or devices leverage persistent catalytic activity of nanozymes, offering a dual benefit of addressing disease-related issues and mitigating the foreign body response elicited by the implants or devices. The inherent properties of nanomaterials further enhance the mechanical behaviour of implants or devices, bestowing them with additional functionalities. This innovative approach holds promise in advancing the field of implants and devices, providing solutions beyond conventional pharmaceutical interventions.

Results

Sufficient characterizations of nanozymes are a prerequisite for their applications. In this section, techniques to characterize physico-chemical, enzymatic and biological properties of nanozymes are discussed (Fig. 4).

Physico-chemical characterization

Size and morphology. The size and morphology of nanozymes affect the number and reactivity of their active sites. Smaller nanozymes offer a higher surface area per unit of volume, resulting in a greater number of active sites[82]. This leads to enhanced activity when compared with larger nanozymes at the same mass concentration. The morphology of nanozymes is equally important, as it influences not only the specific surface area and the quantity of active sites but also the nature of the bonds and reactivity of individual active sites[43,83]. Different crystalline planes exposed on the surface lead to variations in reactivity and catalytic behaviour. To assess the characteristics, various analytical techniques are employed. Dynamic light scattering (DLS) is used to determine the hydrodynamic diameter of nanozymes based on the Stokes–Einstein equation and to evaluate their dispersity by analysing the size distribution. Scanning electron microscopy (SEM) and TEM are utilized to visualize nanozymes at the nanoscale, providing insights into their size and morphology (Fig. 4a). For example, high-resolution TEM can reveal structural details, such as core–shell structures adorned with organic coatings or diverse components, which proves successful modification of nanozymes.

Zeta potential. Surface charge plays a pivotal role in various aspects of nanozymes, including their dispersity, substrate adsorption and targeted delivery. Zeta potential represents the surface charge of nanoparticles in a solution. It is measured using techniques such as laser Doppler electrophoresis (Fig. 4a). Nanozymes with a higher absolute zeta potential value often exhibit superior dispersity. A higher value indicates a stronger electrostatic repulsion between the nanozymes, preventing them from agglomerating and leading to better stability in solution. The activity and targeting ability of nanozymes are also influenced by zeta potential as discussed above[84].

Composition and structure. The composition of nanozymes impacts their active sites. Techniques such as doping and alloying are used to adjust the composition and, consequently, the catalytic activity of nanozymes[85,86]. Several analytical methods are applied to assess the material synthesis and composition modulation. In this regard, inductively coupled plasma (ICP) serves as a quantitative tool to measure the elemental composition of materials. Additionally, X-ray photoelectron spectroscopy (XPS) is a non-destructive method that provides detailed information about the elemental composition, chemical states and electronic states on the surface. Nanozymes with similar compositions but different structures can exhibit substantially varying catalytic activities, requiring further methods to determine their structures. XRD can be used to identify crystalline phases and composition, whereas Fourier transform infrared (FTIR) can identify functional groups on the surface of nanozymes and their molecular conformations. Nuclear magnetic resonance (NMR) is used to determine the chemical environment, such as for MOF-based nanozymes, circular dichroism is used for assessing chiral structures, X-ray absorption fine structure (XAFS) is used for exploring atomic arrangements within the material (especially for coordination environment, bond distances and bond angles for analysing active sites of nanozymes) and high-angle annular dark-field scanning TEM is used for element mapping for atom visualization (Fig. 4a).

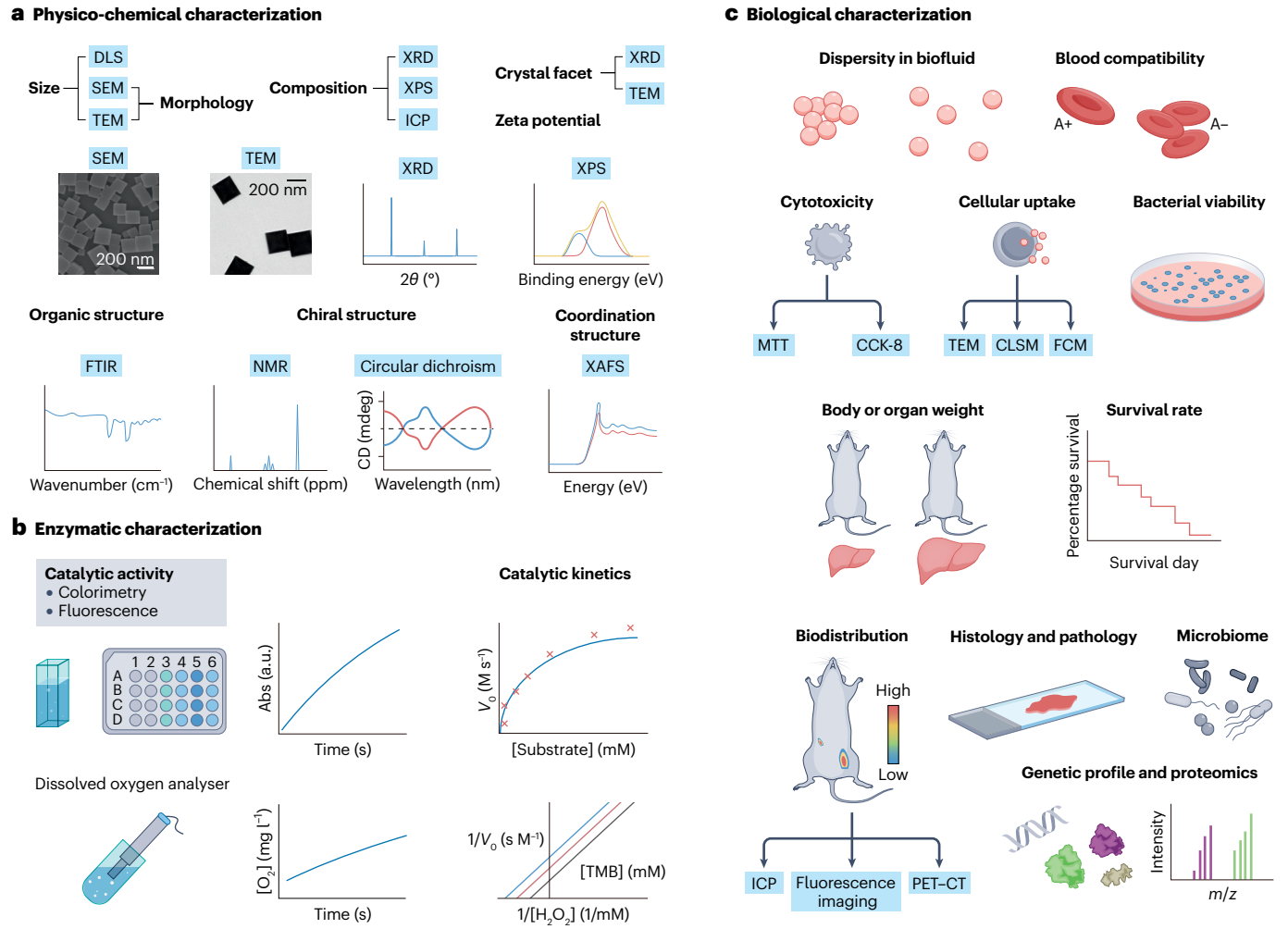

Fig. 4 | Characterization of nanozymes for nanohealthcare. a, Physico-chemical characterization encompasses a range of techniques, including dynamic light scattering (DLS), scanning electron microscopy (SEM), transmission electron microscopy (TEM), X-ray powder diffraction (XRD), X-ray photoelectron spectroscopy (XPS), inductively coupled plasma (ICP), Fourier transform infrared (FTIR), nuclear magnetic resonance (NMR), circular dichroism and X-ray absorption fine structure (XAFS). These techniques analyse various aspects of the nanozymes, such as nanoparticle size, morphology, zeta potential, composition, crystal facet and structural features, including surface modifications and active sites. Representative SEM and TEM images of Prussian blue nanozyme are demonstrated. **b**, Enzymatic characterization involves the study of catalytic kinetics and analysis. This includes assessing the catalytic efficiency, substrate specificity, reaction rates and other parameters that provide insights into the enzymatic activity. **c**, Biological characterization contains both in vitro and in vivo studies. In vitro studies extend to the cellular and bacterial levels. Cellular assessments include the evaluation of cytotoxicity (methylthiazolyldiphenyl-tetrazolium bromide (MTT) and cell counting kit-8 (CCK-8)), examination of cellular uptake (TEM, confocal laser scanning microscopy (CLSM) and flow cytometry (FCM)), and exploration of various cellular functions influenced by nanozymes. Bacterial studies delve into the impact of nanozymes on bacterial viability. In vivo studies include observing the behaviours of nanozymes within living organisms and assessing their influence on pathology, genetic profile, proteomic changes, microbiome and biodistribution, providing insights into their systemic effects. PET–CT, positron emission tomography and computed tomography; TMB, 3,3′,5,5′-tetramethylbenzidine; V_0, initial velocity.

Enzymatic characterization

Catalytic activity. Catalytic activity relates to the rate at which a nanozyme accelerates a chemical reaction. It is quantified using the nanozyme activity unit (U), where 1 U represents the quantity of nanozyme required to catalyse the transformation of 1 µmol of substrate per minute under optimal reaction conditions[87]. The specific activity is the measure of nanozyme activity units per milligram of the nanozyme. The turnover frequency represents the number of substrate molecules converted by an active site per unit of time[88,89]. Turnover frequency compares activities based on the normalized active sites rather than the whole of nanozymes, focusing on the sites themselves and excluding extra factors such as sizes and unexposed atoms of the nanozymes.

To evaluate the catalytic activities of oxidase-like and peroxidase-like nanozymes, a UV–Vis spectrophotometer or a microplate reader is used to monitor the absorbance change in oxidation or peroxidation of reductive substrates over time, including TMB, ABTS and 3,3′-diaminobenzidine (DAB)[12] (Fig. 4b). Similarly, hydrolase-like

nanozymes often use *p*-nitrophenyl phosphate or bis(*p*-nitrophenyl) phosphate as substrates. The product *p*-nitrophenol has a characteristic absorption peak that can be measured using a spectrometer[90]. For the activity of catalase-like nanozymes, a dissolved oxygen analyser can be used to detect the production of oxygen during the catalytic process (Fig. 4b). This method is limited by a narrow detection range, owing to a low saturated concentration of dissolved oxygen. Alternative methods are to measure oxygen pressure signals in a confined space (such as in a confined tube)[91], or to utilize oxygen-sensitive probes. For example, dopamine is an oxygen-sensitive probe, whose self-polymerization can be activated by O_2 produced in catalase reactions and produce colour[29]. For superoxide dismutase-like nanozymes, several fluorescent or colorimetric probes can be oxidized by superoxide radical ($^{\bullet}O_2^-$) to assess activities, including dihydroethidium, nitrotetrazolium blue chloride, iodonitrotetrazolium chloride, water-soluble tetrazolium salt (WST-8) and cytochrome *c*[30]. The choice of probes depends on their physicochemical properties and those of nanozymes. For example, the limited water solubility of the reduced by-product of nitrotetrazolium blue chloride can restrict the sensitivity of detection. Furthermore, the dihydroethidium probe can be oxidized by nanozymes with oxidative abilities providing false negative results. In addition, electron paramagnetic resonance is a more reliable method to analyse superoxide dismutase-like nanozymes, through the use of 5,5-dimethyl-1-pyrroline-*N*-oxide to indicate the presence of $^{\bullet}O_2^-$ (ref. 92).

Catalytic kinetics. Catalytic kinetics describes the effect of physicochemical factors on the catalysed reaction rate, including temperature, pH, activator, inhibitor, nanozyme concentration and substrate concentration[93]. By studying the kinetics, the reaction environment a nanozyme is mostly adapted to and where it could be applied can be determined, as the detection, diagnosis or therapy may offer different environmental conditions for reactions. Based on kinetics, catalysis mechanisms can be studied and nanozymes with high activity applied in specific situations can be further modulated or rationally designed[94].

Similar to enzymes, the initial velocity (V_0) of a nanozyme-catalysed reaction related to a substrate concentration mostly obeys the Michaelis–Menten equation based on the assumption of steady-state equilibrium. In this context, two parameters — maximum velocity (V_{max}) and the Michaelis constant (K_m) — are obtained to characterize the catalytic kinetics of nanozymes. V_{max} is the maximal velocity of a catalytic reaction by a saturated nanozyme under given conditions. However, V_{max} depends on the nanozyme concentration, making it hard to compare across samples. To overcome this, the catalytic constant ($k_{cat} = V_{max}$/[Nanozyme]) is used to compare velocities among different nanozymes at normalized nanozyme concentration. K_m refers to the substrate concentration that gives $V_{max}/2$, at which half the active sites of the nanozyme function. Generally speaking, the K_m value describes a nanozyme's affinity towards its substrates — a lower K_m value indicates a higher affinity. Combining the k_{cat} and K_m values gives the specificity constant (k_{cat}/K_m) to determine the catalytic efficiency of the nanozyme (Fig. 4b).

The kinetics of reactions catalysed by oxidase-like, catalase-like or hydrolase-like nanozymes can be assessed directly by the Michaelis–Menten equation as they have only one substrate (or their second substrate concentration remains constant). Their initial velocities are measured using a spectrometer or dissolved oxygen analyser. For two-substrate reactions catalysed by peroxidase-like nanozymes, the standard assay is to keep one substrate concentration fixed as a control variable and test it as a one-substrate reaction using a spectrometer[27]. Apparent K_m and V_{max} values vary with different fixed concentrations, which allows the kinetic mechanisms of peroxidase-like nanozymes, such as ping-pong mechanisms or sequential mechanisms, to be explored[16]. In a catalytic reaction that follows a ping-pong mechanism (such as A + B → C + D), substrate A first binds a nanozyme to form a binary complex. The complex reacts to convert substrate A to product C and the nanozyme to its intermediate state. After product C is released, substrate B binds the intermediate state of nanozyme to yield product D and turn the nanozyme into the original state. In sequential mechanisms, both substrates bind the nanozyme to form a ternary complex in a random or ordered binding sequence. The two products are released to regenerate the nanozyme. Note that there is no convenient method to detect the catalytic kinetics of superoxide dismutase-like nanozymes because $^{\bullet}O_2^-$ is so unstable that it is frequently necessary to employ stopped-flow spectrophotometry to measure concentration changes within extremely brief temporal intervals[95,96].

Biological characterization

Interaction between nanozymes and biofluids. Understanding the interaction between nanozymes and biofluids is important in the biological characterization of nanozymes[97]. Typically, a set of artificial solutions, designed to mimic the composition and conditions of physiological environments, are used to evaluate the nanozymes[84,98]. In simulated solutions, nanozymes may exhibit aggregation when interacting with components present in the solution, owing to the high specific surface energy inherent in nanomaterials. These unwanted interactions significantly impact the activities of designed nanozymes, subsequently altering the functionalities of these nanozymes.

Nanozymes have the potential to impact blood stability; haemolysis tests should be conducted to prevent the breakdown of blood cells in the body[99,100]. Materials with a high degree of compatibility with blood are selectively utilized in these scenarios. The impact of nanozymes on blood function is multifaceted and depends on various factors, including their physico-chemical properties, surface modifications and intended applications. The main approaches to evaluate blood functions are routine blood examination and blood biochemical examination. For example, the anti-oxidant nanozymes can mitigate oxidative stress in the bloodstream by scavenging ROS, which contributes to the redox balance of blood. The results of routine blood examination (for example, leukocyte count) can reveal the impact of nanozymes on blood functions. Moreover, in anticoagulation research, careful consideration must be given to the interplay between platelets and nanozymes.

In vitro studies. For in vitro studies, nanozyme catalytic abilities within a suitable cellular model should be evaluated to reflect potential therapeutic effects. The initial step entails selecting a suitable cell line for the study, typically choosing cells at the diseased sites. For instance, in vascular diseases, cell lines such as human umbilical vein endothelial cells and vascular smooth muscle cells are commonly employed to simulate a vascular enviroment[101]. Once the cell types are chosen, cytotoxicity is assessed using assays such as the methylthiazolyldiphenyl-tetrazolium bromide (MTT) colorimetric assay or the cell counting kit-8 (CCK-8) assay. High absorbance of the cell viability-related product indicates low toxicity of nanozymes. During these assays, factors such as overlapping of absorbance between chromogenic substrates and nanozymes should be considered, especially for carbon-based materials that are hard to wash away, as well as the interaction between the reagents for MTT or CCK-8 and nanozymes. Nanozymes, such as graphene-based

materials, can react with these colorimetric substances, resulting in false positive signals[102]. The cellular uptake of nanozymes can be studied using techniques such as TEM, confocal laser scanning microscopy (CLSM) and/or analytical flow cytometry (FCM) to determine whether nanoparticles are successfully internalized.

After cell viability and uptake assessments, various cellular models are designed to better mimic in vivo scenarios to determine the efficiency of nanozymes. For instance, to evaluate the ability of nanozymes to traverse physiological barriers such as the blood–brain barrier, transwell experiments are employed to simulate the mass transfer process between specific cell lines[103]. For malignant tumours, multicellular tumour spheroids can be used to mimic the acidic and enzyme microenvironments of solid tumours[104]. Implants or devices incorporating nanozymes can serve as a platform for investigating proliferation and migration of cells, assessed through SEM or fluorescent imaging[105,106]. To visualize and quantify outcomes post treatment, fluorescence signals (utilizing fluorescence microscopy and FCM) and microscale imaging (TEM and SEM) prove effective[107]. These technologies aid in evaluating the behaviours of specific cells, and initial results obtained in vitro can provide insights into potential outcomes when applied in vivo.

Bacterial behaviour and viability are key factors in microbial diseases. Monitoring bacterial viability often involves measuring the optical density at 600 nm after co-incubation with nanozymes. The optical density value at 600 nm is sensitive to changes in bacterial cell concentration during growth[108]. At this wavelength, bacterial cells scatter and absorb light, and the amount of light absorbed is proportional to the number of cells present. Additionally, the spread plate method is applied to quantify colony-forming units of bacteria, further contributing to the assessment of bacterial states. Nanozymes exhibit a dual nature against bacterial infections. On the one hand, they function as robust ROS producers, deploying this capability as a potent mechanism to counteract bacterial invasions[109]. On the other, nanozymes can be ingeniously engineered to serve as guardians, shielding probiotics from the deleterious effects of ROS, thereby presenting a versatile tool in microbial control scenarios[98].

Biofilm formation is a common bacterial strategy to resist external stimuli, leading to recurrent infections[109]. The evaluation of nanozymes often involves studying their ability to eliminate biofilm or prevent biofilm formation. Methods such as crystal violet staining and fluorescent dyes prove valuable in visualizing the status of biofilms[53].

In vivo studies. Typically, the choice of animal models hinges on the specific diseases under investigation. Rodents, such as mice, and mammals, including rabbits, dogs and pigs, are typical animal models for evaluating nanozyme efficacy. Rabbits serve as ideal models for eye diseases, owing to the similarities in size and structure of their eyes compared with humans. This resemblance allows researchers to draw pertinent insights into performance of nanozymes within ocular health[110]. Similarly, when exploring diseases of the intestines, mice of the C57BL/6 strain and Beagle dogs emerge as recognized subjects, specifically for studying bowel inflammation[98]. In cases where gene manipulation is required to emulate genetic deficiencies, such as in atherosclerosis studies, unique mouse models such as apolipoprotein E-deficient mice are employed. These genetically engineered mice are incapable of producing apolipoprotein, leading to fat accumulation, particularly after a high-fat diet[101]. With the continuous evolution of gene technology, researchers have the chance to develop an ever-expanding array of animal models, each tailored to their specific research needs.

Once the appropriate animal models have been screened and administration routes have been selected, in vivo studies can commence. The key here lies in determining the optimal dosage and timing, which are essential for effective therapeutic outcomes. In the later stages of animal experiments, various approaches are employed to comprehensively assess the efficacy of nanozymes. Certain visualization technologies aid in the initial assessment of efficacy. For instance, colonoscopy allows for in situ observation of the intestine in cases of inflammatory bowel disease (IBD)[98], ultrasound technology facilitates imaging observation of vascular-related diseases[111] and nuclear magnetic technology enables the observation of tumours and other diseases through visualization[112]. Collecting histological and pathological data is paramount; techniques such as immunofluorescence, immunohistochemistry and pathological section staining offer invaluable insights into the physiological and structural changes within the tissues. Furthermore, gaining an in-depth understanding often necessitates the analysis of genetic profiles and proteomics, offering a deeper glimpse into the underlying molecular mechanisms and signal pathways affected by nanozymes. In cases of diseases related to microbial factors, an exploration of the microbiome is indispensable, unravelling the interplay between microorganism and host before and after treatment[113]. Post-treatment evaluations are a vital component of the research process, encompassing parameters such as survival rates, body or organ weights and cognitive abilities[114]. These metrics help evaluate the impact and effectiveness of nanozymes.

Lastly, the often underestimated aspect of nanozymes is biodistribution. To address this, techniques such as ICP are used to detect the content of nanozymes in specific organs. In cases where nanozymes can be tagged with radionuclides or fluorescent dyes, their distribution is visually elucidated through imaging modalities such as positron emission tomography and computed tomography (PET–CT) scans or fluorescent imaging[115]. Beyond these, body fluids and excrement can serve as valuable analytes, shedding light on the metabolism and elimination processes of nanozymes[26,116].

Applications

In this section, the application of nanozyme-based nanohealthcare in several key areas is discussed with some representative examples.

In vitro and in vivo diagnostics

Nanozyme-involved reactions. Nanozymes catalyse reactions that are indicative of certain diseases. The signal indicating a disease condition is often based on the alteration of reaction substrates or the appearance/disappearance of reaction products. Various types of signals can be employed for detection, including colorimetric, fluorescent and surface-enhanced Raman scattering signals. These signals are generated as a consequence of nanozyme catalysis[117–119] (Fig. 5a).

Peroxidase-like or oxidase-like nanozymes have gained attention, owing to their ability to oxidize colourless substrates such as TMB to produce coloured products in the presence of H_2O_2 or O_2. The intensity of the colorimetric signal generated is directly related to the concentration of reactants, such as H_2O_2. In some cases, the targeted molecules (for example, glutathione (GSH)) might react with H_2O_2 or reduce the coloured product[120]. In such assays, the colorimetric signal and the concentration of the reducing target molecule can be used to determine the target's concentration. The ability of nanozymes to catalyse reactions and their sensitivity to changes in the presence or concentration of target molecules has been utilized for the detection and quantification of specific analytes or biomolecules[121–123]. In addition, blocking

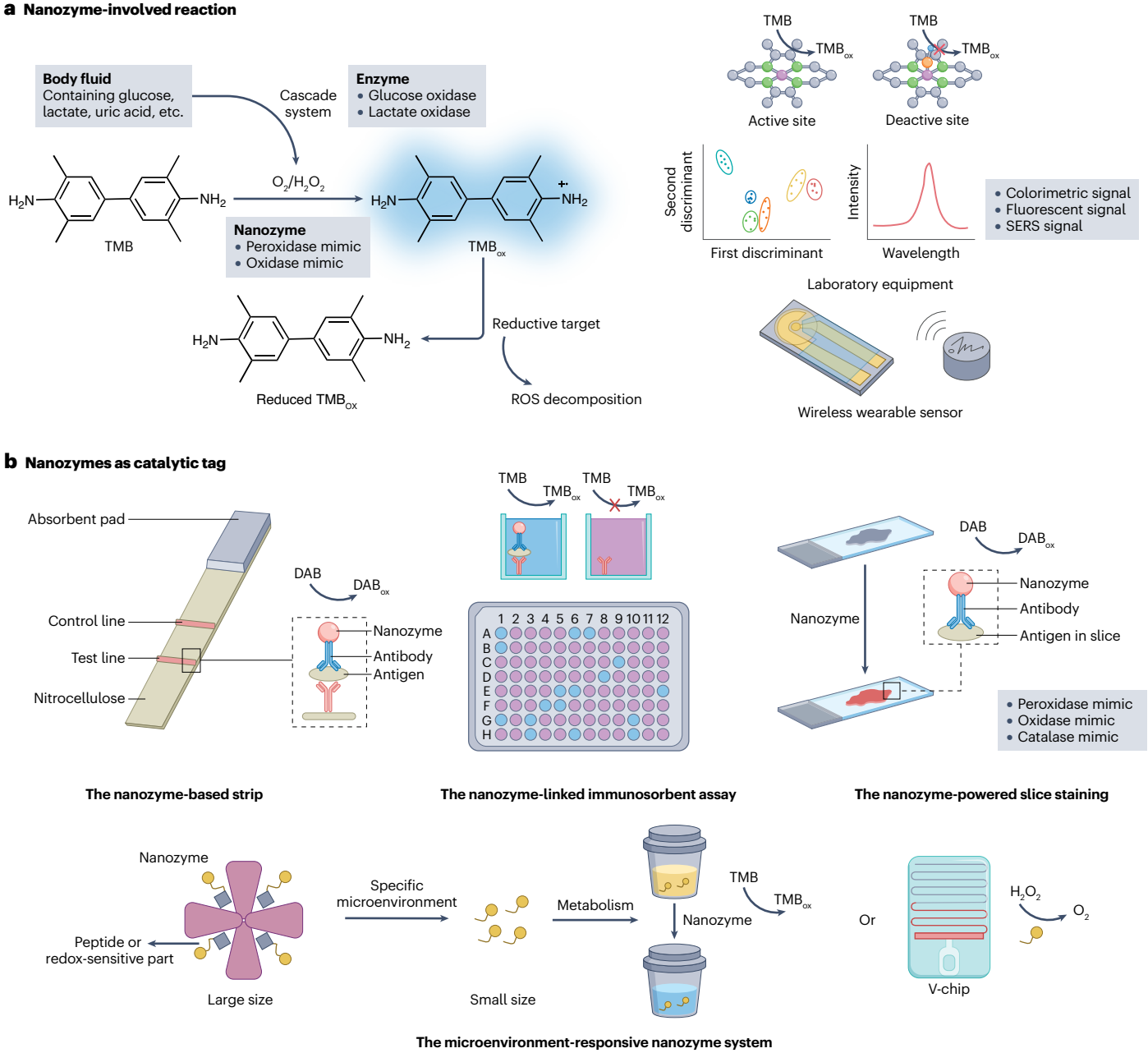

Fig. 5 | Nanozyme-based diagnosis and representative applications. **a**, In nanozyme-involved reactions, the focus is the active participation of nanozymes in detection processes. Nanozymes, acting as peroxidase or oxidase mimics, catalyse the conversion of targets into easily detectable signals. The peroxidase-like catalysis is facilitated by H_2O_2 generated through enzyme catalytic reactions. Factors influencing the detectable signal can offer alternative ways to detect substances that may block the active sites or reduce the oxidized product/H_2O_2. The sensing signal can be absorbance and other visualization approaches. **b**, Nanozymes, as catalytic tags, rely on recognition processes facilitated by surface-conjugated ligands, such as antibodies. Nanozymes enhance sensing signals catalytically within various technologies. Nanozymes are integral to the development of diagnostic strips for virus detection. Specific interactions between antibodies and antigens enable rapid and accurate detection using nanozyme-based strips. The nanozyme-linked immunosorbent assay operates similarly, utilizing surface-conjugated components for disease marker detection. Nanozymes can be used to stain pathological tissue sections. With the assistance of antibodies, nanozymes target specific pathological regions, catalysing chromogenic substrates (such as 3,3′-diaminobenzidine (DAB)) to their oxidized state to reveal the disease status. For in vivo diagnostics, nanozyme systems can be triggered by the microenvironment, responding to elevated levels of reactive oxygen species (ROS) and dysfunctional protease levels. This triggers decomposition into metabolizable fragments that can be excreted through the kidneys. Nanozymes present in urine can oxidize chromogenic substances or decompose H_2O_2 into oxygen, allowing for microfluidic monitoring of diseases. SERS, surface-enhanced Raman spectroscopy; TMB, 3,3′,5,5′-tetramethylbenzidine.

the active sites prevents nanozymes from catalysing the oxidation reaction, also resulting in a reduced signal, where the change in signal intensity reflects the concentration of target molecules. For example, the active site structure similarity allows the single-atom nanozyme to serve as a substitute for cytochrome P450 in assessing the potential toxicity of drugs and evaluating drug metabolization. During these processes, the active site of single-atom nanozymes is blocked or regenerated, indicating the interaction between enzymes and drugs[124]. The use of nanozymes in drug evaluation offers a versatile avenue for enhancing the safety and efficacy assessment of pharmaceutical compounds.

On the other hand, the lack of selectivity in nanozymes may limit their specificity in complex biological environments. In such cases, combining nanozymes with enzymes to form a cascade system presents a promising solution. In a cascade system where enzymes work in conjunction with high-performance nanozymes, a sequential transformation of a target molecule into an easily detectable substance, such as H_2O_2, can be made. Once H_2O_2 is produced, the nanozymes can then detect and quantify the concentration of H_2O_2, thereby indicating the level of the target molecule. For example, by integrating haemin and glucose oxidase (GOx) into the ZIF-8 nanostructure, an integrated nanozyme (INAzyme) can achieve glucose detection through a colorimetric cascade approach. H_2O_2 produced in the GOx catalytic process can serve as the next peroxidase-mimicking substrate. Such an INAzyme-based colorimetric assay could easily detect cerebral glucose changes in the microdialysed samples from the brain of living rats. Furthermore, considering the significant therapeutic and scientific importance in practical applications, microfluidic technology was introduced to form an online in vivo analytical platform, which can monitor the dynamic changes of glucose in acute rat brain insults (for example, ischaemic stroke and head trauma)[21]. Nanozymes, together with lactate oxidase to form a cascade system to identify lactate, find applications in wearable sensor technology. An in-ear integrated array has been developed for in situ monitoring in various health-related contexts related to the brain, such as injury and stress. In this system, the nanozyme, particularly Prussian blue, acts as a converter, facilitating the conversion of H_2O_2 to electronic signals. When combined with lactate oxidase, this integrated array enables real-time health monitoring, providing insights into brain-related conditions[125]. The use of cascade systems in wearable sensors holds promise for advancing personalized and continuous health monitoring.

Nanozymes as catalytic tags. Nanozymes can be integrated into existing diagnostic techniques as catalytic tags to enhance sensitivity, specificity and accuracy, making them more effective in identifying diseases or specific biomarkers (Fig. 5b).

Specific recognition elements, such as antibodies, have been used to modify nanozymes[126]. The modified nanozymes, which are specifically linked to the targets, can be employed for enrichment and concentration to improve the sensitivity of detection. The addition of chromogenic substrates or other detection reagents initiates a reaction to generate signals corresponding to the level of the captured biomarkers. For example, an Fe_3O_4 magnetic nanozyme-based immunochromatographic strip (nanozyme strip) has been developed to detect Ebola virus. The surfaces of nanozymes were coated with antibody to improve their selectivity. Comparable with ELISA in accuracy, a nanozyme strip is simpler (without special equipment) and has a faster detection time. Based on its sensitivity and simplicity, the nanozyme strip emerges as a crucial screening tool for Ebola-stricken areas[34]. Nanozymes can also be used to assist with slice staining. Iron oxide nanozymes were encapsulated inside a recombinant human heavy-chain ferritin protein shell to form magnetoferritin nanoparticles, which can be used to target and visualize tumour tissues. In testing of 474 clinical specimens from nine cancer types, these nanozymes achieved 98% sensitivity and 95% specificity in distinguishing cancerous from normal cells, which showcases great promise for clinical use[74].

Nanozyme-based sensors or detectors can be designed to respond to specific biomarkers. Ultra-small gold nanoclusters prove promising for in vivo imaging with exceptional tumour accumulation and efficient renal clearance. Their intrinsic peroxidase-like activity enables the design of multifunctional protease nano-sensors, offering a direct colorimetric urinary read-out of disease states. The protease-sensitive complex is cleaved under the relevant dysregulated proteases and the liberated gold nanoclusters are filtered into urine for diagnostics. This nano-sensor showcases a versatile approach for rapidly detecting various diseases with specific enzymatic signatures[26]. For point-of-care analysis, the catalase-like capabilities of platinum nanozymes can be integrated into a companion volumetric bar-chart chip (V-Chip) system[116]. The changes induced in nanozyme systems owing to biomarker interaction provide a clear and specific signal that allows for the sensitive detection and quantification of the targets.

The versatility of nanozymes in generating detectable signals via diverse reaction pathways and assisting established diagnostic tests has influenced disease detection and monitoring. Their application in these diagnostic methods offers the potential for rapid, sensitive and specific detection of diseases, contributing significantly to the advancement of personalized medicine and point-of-care diagnostics.

Nanozyme-based therapeutics

In this section, the applications of nanozyme-based therapy in several diseases are discussed with some representative examples (Fig. 6).

Neurodegenerative disease. Oxidative stress is vital in the development of various neurodegenerative diseases, contributing to neuronal death and functional impairment in the nervous system. Protein oxidation, such as the case of amyloid-β peptide (Aβ) interfering with metal utilization in the brain, is linked to the pathogenesis of neurodegenerative disorders (Fig. 6a). Several types of nanozymes, including fullerenes[127], Mn_3O_4 nanoflowers[83], CeO_2 (ref. 128) and Prussian blue[129], have been developed and studied in animal models for neurological disorders. C_{60}-C_3, a derivative of fullerene, has been shown to treat Parkinsonian non-human primates[127]. These nanozymes exhibit the ability to mitigate neurodegenerative diseases by inhibiting pyroptosis. Nanozymes such as Prussian blue can act as inhibitors of pyroptosis by eliminating ROS, thereby reducing the progression of diseases such as Parkinson disease and neurodegeneration in mice. These nanozymes achieve treatment by inhibiting the activation of NLRP3 inflammasome, downregulating the cleavage of gasdermin D and reducing the production of inflammatory factors[129]. Beyond scavenging excessive ROS, nanozymes equipped with hydrolytic activity (such as polyoxometalate-based material) can degrade pathogenic proteins, addressing the multifaceted neurotoxicity associated with Aβ[103,130,131]. Through proteolysis, nanozymes can mitigate Aβ-mediated toxicity by clearing Aβ and reducing cellular ROS levels. This multifunctional ability holds promise for addressing the complex mechanisms underlying neurodegenerative diseases.

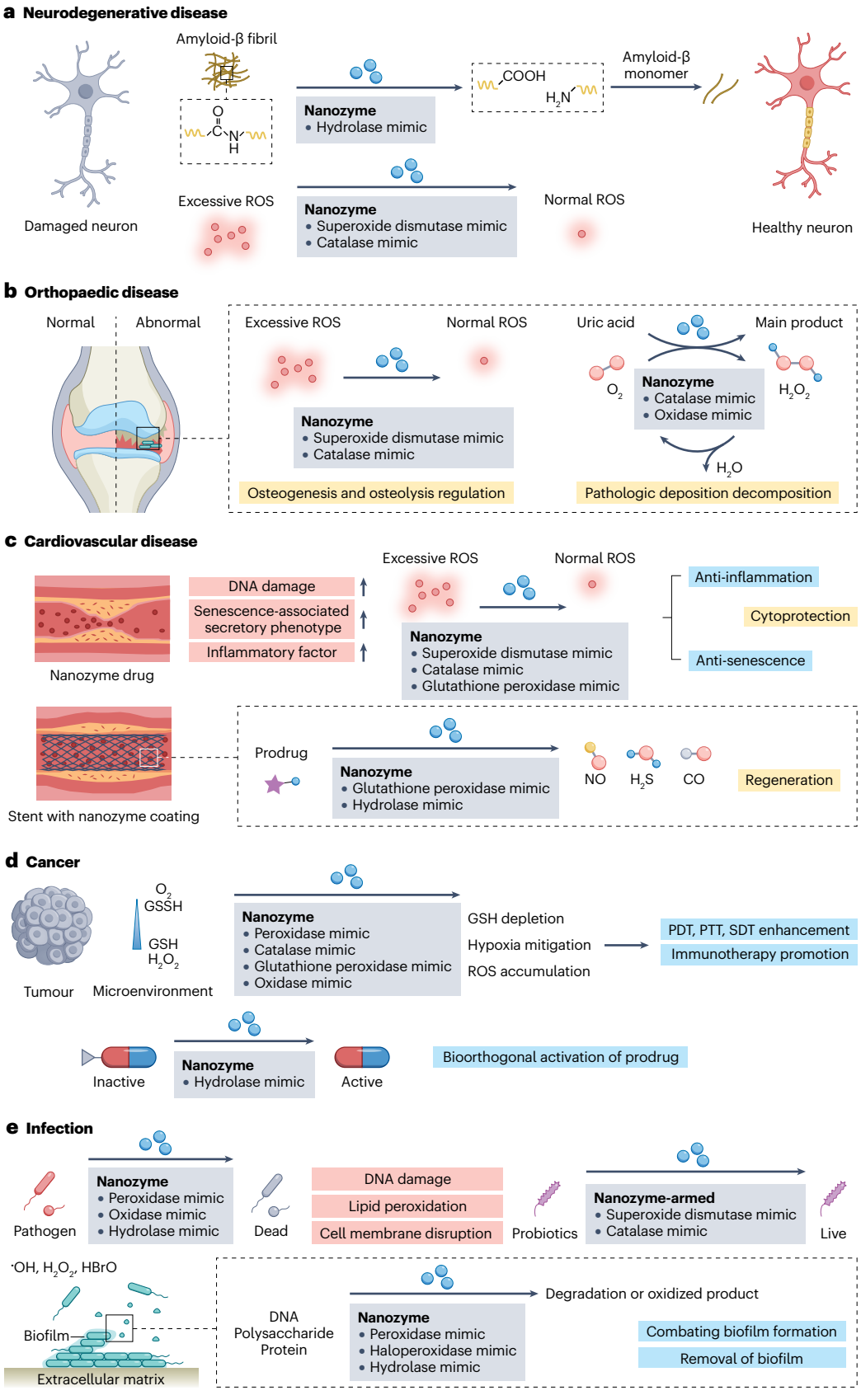
a Neurodegenerative disease
Amyloid-β fibril
Damaged neuron
Nanozyme
• Hydrolase mimic
COOH
H2N
Amyloid-β monomer
Healthy neuron
Excessive ROS
Nanozyme
• Superoxide dismutase mimic
• Catalase mimic
Normal ROS
b Orthopaedic disease
Normal
Abnormal
Excessive ROS
Normal ROS
Nanozyme
• Superoxide dismutase mimic
• Catalase mimic
Osteogenesis and osteolysis regulation
Uric acid
Main product
O2
H2O2
Nanozyme
• Catalase mimic
• Oxidase mimic
H2O
Pathologic deposition decomposition
c Cardiovascular disease
Nanozyme drug
DNA damage
Senescence-associated secretory phenotype
Inflammatory factor
Excessive ROS
Normal ROS
Nanozyme
• Superoxide dismutase mimic
• Catalase mimic
• Glutathione peroxidase mimic
Anti-inflammation
Cytoprotection
Anti-senescence
Stent with nanozyme coating
Prodrug
Nanozyme
• Glutathione peroxidase mimic
• Hydrolase mimic
NO
H2S
CO
Regeneration
d Cancer
Tumour
O2
GSSH
GSH
H2O2
Microenvironment
Nanozyme
• Peroxidase mimic
• Catalase mimic
• Glutathione peroxidase mimic
• Oxidase mimic
GSH depletion
Hypoxia mitigation
ROS accumulation
PDT, PTT, SDT enhancement
Immunotherapy promotion
Inactive
Nanozyme
• Hydrolase mimic
Active
Bioorthogonal activation of prodrug
e Infection
Pathogen
Nanozyme
• Peroxidase mimic
• Oxidase mimic
• Hydrolase mimic
Dead
DNA damage
Lipid peroxidation
Cell membrane disruption
Probiotics
Nanozyme-armed
• Superoxide dismutase mimic
• Catalase mimic
Live
·OH, H2O2, HBrO
Biofilm
Extracellular matrix
DNA
Polysaccharide
Protein
Nanozyme
• Peroxidase mimic
• Haloperoxidase mimic
• Hydrolase mimic
Degradation or oxidized product
Combating biofilm formation
Removal of biofilm

Fig. 6 | Nanozyme-based therapeutics demonstrate versatile applications across various diseases. a, In neurodegenerative disorders, nanozymes function as reactive oxygen species (ROS) scavengers, eliminating excessive ROS. Protease-mimics contribute to the removal of amyloid-β peptide (Aβ) fibrils and repression of Aβ aggregation. **b**, In orthopaedic disease, nanozymes with anti-oxidant and hydrolase-like abilities regulate osteogenesis and osteolysis. Besides, uricase mimics decompose pathologic depositions. **c**, For cardiovascular disease (CVD), anti-oxidant nanozymes achieve anti-inflammation and anti-senescence, particularly in atherosclerosis. They also serve as functional coatings on implants, catalysing prodrugs to produce beneficial molecules such as CO, NO and H_2S, promoting vascular regeneration. **d**, In cancer, nanozymes modulate the tumour microenvironment, deplete glutathione (GSH), accumulate ROS and mitigate hypoxia, enhancing treatment tactics such as photodynamic therapy (PDT), photothermal therapy (PTT), sonodynamic therapy (SDT) and immunotherapy. Nanozymes also assist with in situ drug production using hydrolase-like activity. **e**, In infections, nanozymes act as pathogenic killers or probiotic guardians, leveraging their diverse abilities. They also produce toxic substances such as free radicals, which destroy extracellular matrices and interfere with quorum sensing signalling molecules to eliminate biofilms and inhibit biofilm formation. GSSH, oxidized glutathione.

Orthopaedic disease. Orthopaedic disease encompasses a spectrum of conditions such as inflammatory arthritis, osteoporosis, traumatic bone defects, bacteria-associated infections and osteosarcoma[39]. Nanozymes have emerged as a promising avenue in effectively addressing these orthopaedic diseases, owing to their remarkable anti-oxidant ability and regulation of related signalling pathways such as inflammation, osteogenesis and osteolysis (Fig. 6b).

In osteoarthritis characterized by pathological joint deterioration, oxidative stress induced by elevated ROS disrupts joint microenvironment homeostasis, leading to lipid peroxidation, DNA damage and an intensified inflammatory response. ROS-scavenging nanozymes can mitigate oxidative stress in the pathogenesis of osteoarthritis. For instance, by attenuating oxidative stress, Prussian blue nanozymes can effectively remodel the joint microenvironment and then protect chondrocytes, delaying the progression of osteoarthritis[132]. Gout, the most common inflammatory arthritis, occurs when there is excessive uric acid agglomeration. In this case, uric acid degrading combined with ROS scavenging is an ideal method to cure this disease. The nanozyme with uricase-like and catalase-like activities (such as Pt/CeO_2 composite) was developed for alleviating acute gout. Uricase mimics decompose uric acid, whereas catalase mimics eliminate the by-product H_2O_2 generated from uric acid degradation[22].

In osteogenic therapies, osteoblastic differentiation is a critical section. Phosphate anions are essential for bone remodelling, making alkaline phosphatase an early marker for osteogenesis[133]. Anti-oxidative nanozymes such as ceria can upregulate the expression of anti-inflammatory cytokines and promote mesenchymal stem cell proliferation[134]. Meanwhile, because of ROS scavenging, ceria can promote osteogenic differentiation to improve the alkaline phosphatase activities in mesenchymal stem cells[135].

Cardiovascular disease. Cardiovascular disease (CVD) represents a group of disorders affecting the heart and blood vessels. Chronic inflammation serves as the primary pathological mechanism underlying CVD, making it a focal point for therapeutic interventions. Nanozymes can treat CVD via two primary mechanisms[136]. First, nanozymes can directly scavenge excess ROS. This action modulates the inflammatory microenvironment, thereby attenuating the progression of CVD. Second, nanozymes can catalyse the production of signal molecules, facilitating communication among cells in the pathological environment, promoting therapeutic effects (Fig. 6c).

In ischaemia–reperfusion injury, nanozymes with mitochondrion-targeting capabilities prove effective in mitigating mitochondrial oxidative damage[37]. This targeted approach aids in the recovery of cardiac function. Ischaemic stroke, characterized by a sudden blockage of blood supply to the brain, leads to an explosive increase in ROS and neural apoptosis during cerebral ischaemic–reperfusion injury. A cascade nanozyme with multi-enzyme activities (such as selenium-containing MOF-based nanozyme) demonstrates cytoprotective abilities by eliminating intracellular ROS and inhibiting neural apoptosis. This intervention results in a decrease in infarct volume and improved neurological deficits[137].

Atherosclerosis is the primary pathological basis of CVD. In atherosclerosis plaques, where senescent cells and elevated inflammatory microenvironments prevail, nanozymes engineered with anti-senescence and multiple anti-oxidant activities prove effective in therapy. These nanozymes (such as MOF@Se nanozyme) protect DNA from damage, attenuate cell senescence and decrease cellular uptake of oxidized low-density lipoprotein, thereby suppressing foam cell formation[101].

In metabolic conditions associated with CVD, there is a demand for specific gas supplies, such as oxygen and nitric oxide. Under a hypoxic condition, nanozymes can act as an oxygen transfer station to improve the oxygen in this environment. Owing to the activation of hypoxia-inducible factor 1α, angiogenesis is promoted[138,139]. Nanozymes with GPx-like activities catalyse the prodrugs of nitric oxide (for example, *S*-nitrosoglutathione) to nitric oxide, which is an important signal molecule in CVD therapy. For example, copper-based nanozyme-functionalized artificial vessels could promote normal vessel reconstruction by producing nitric oxide. Generating nitric oxide by the copper-based nanozymes, the neointimal hyperplasia in vessels was suppressed and the inflammatory response was reduced[140]. In another application, nanozymes can be coated on a vascular stent. Acting as a nitric oxide producer, nanozyme-coated stents can largely avoid late stent thrombosis and in-stent restenosis[105,141]. Such coating inhibited platelet aggregation and activation via the nitric oxide–cGMP signalling pathway, and significantly reduced thrombosis in an ex vivo extracorporeal circulation model[105].

Cancer. The tumour microenvironment, characterized by hypoxia, acidity and elevated H_2O_2 levels, plays a pivotal role in cancer progression. Nanozymes can improve therapies such as photodynamic therapy (PDT), PTT, sonodynamic therapy (SDT) and chemotherapy by changing the microenvironment of tumours.

The peroxidase-like nanozymes are extensively applied in cancer therapy by catalysing H_2O_2 into $^{\cdot}OH$. For instance, pyrite peroxidase-like nanozymes, owing to their strong affinity for H_2O_2, generate abundant $^{\cdot}OH$, effectively eliminating tumour cells. Additionally, these nanozymes can convert GSH into oxidized glutathione (GSSH), contributing to ferroptosis-based tumour therapy[142]. Exploiting GSH oxidase-like capabilities, copper hexacyanoferrate nanozymes effectively decrease intracellular GSH levels, amplifying their impact through cascade catalysis in collaboration with peroxidase-like or oxidase-like

activities[143]. This combined action increases ROS accumulation, enhancing the anticancer effects of the nanozymes.

Nanozyme catalytic production of O_2 in pathological regions can interrupt the metabolism of cancer cells. Nanozymes can alleviate the hypoxic tumour microenvironment by catalysing H_2O_2 into O_2, thus enhancing therapeutic outcomes that rely on sufficient O_2 levels for their efficacy in cancer treatments. This multifunctional approach showcases the potential of nanozymes in creating a conducive therapeutic environment by regulating oxygen levels, which can be beneficial for further cancer therapy[144,145].

Nanozymes also function in prodrug activation[146]. For instance, a degradable bio-orthogonal nanozyme can enhance the efficiency of mitoxantrone prodrug activation and improve therapy[147,148]. Moreover, nanozymes facilitate the synthesis of cell-selective and subcellular organelle-targeted resveratrol analogues through copper-catalysed azide–alkyne cycloaddition reactions. This approach exhibits promise in tumour therapy[149,150] (Fig. 6d).

Infection. Nanozymes, in contrast to traditional antibiotics, exert their antibacterial effects through catalytic activities[151]. Specifically, nanozymes such as peroxidase mimics and oxidase mimics generate ROS, disrupting bacterial structures, inhibiting growth and leading to bacterial demise[152]. Nanozymes may also possess photothermal effects or magnetic properties. Light irradiation or magnetic fields can exploit these properties, boosting enzyme activity and inducing bacterial death through photothermal or magnetocaloric heating. Nanozymes also offer a promising approach to combat viral and fungal infections[113,153,154].

In addition, nanozymes have shown potential in combating biofilms. For anti-biofilm applications, hydrolase-like activities can target the biofilm matrix. This matrix often contains polysaccharides, proteins and DNA, providing structural support to the biofilm. Nanozymes with broad hydrolase-like activities (such as cerium-based MOFs) can degrade this matrix, making it more vulnerable to other antimicrobial strategies[53]. Besides, ROS-producing activities of nanozymes induce oxidative stress in microbial cells and, finally, disrupt biofilms[155,156].

Beyond their antimicrobial role, nanozymes can shield probiotics from oxidative stress at diseased sites[98,157,158]. Nanozyme-armed probiotics can survive in excess ROS induced by over-proliferation of pathogenic bacteria at diseased sites. By preventing the ROS from probiotics, nanozymes contribute to restoring the balance of the microbial ecosystem (for example, intestinal microbiota), offering an avenue for disease treatment. This dual functionality positions nanozymes as versatile agents capable of not only eliminating harmful microorganisms but also safeguarding beneficial microorganisms. This innovative approach holds promise for preserving the delicate balance of microbial communities in disease contexts (Fig. 6e). Furthermore, nanozymes have the potential to modulate the release of enzymes involved in bacterial metabolism and the metabolites produced by bacteria[159–161].

Reproducibility and data deposition

Factors affecting reproducibility

There are several factors influencing the activities of nanozymes, which are critical for the reproducibility and reliability of nanozyme-based research.

Nanozyme synthesis. Minor variations in the synthesis conditions may profoundly influence the properties of nanozymes. Therefore, the control of synthetic parameters is necessary. Researchers provide increasingly detailed information, including temperature, reaction time and even stirring speed, but still face challenges in enabling consistent replication of results. Moreover, the purity of reagents is critical. Consistent sourcing from the same chemical supplier is advisable. Additionally, removing excessive reagents that are not consumed in reaction after synthesis is essential to obtain pure samples for reliable characterization.

Enzymatic evaluation. There are various methods to evaluate nanozyme activities, such as superoxide dismutase-like activities. This diversity in assessment methods brings challenges in comparing different nanozymes effectively. A comprehensive and comparative study for the methods of superoxide dismutase-like activities has been published, which may offer assistance for verification of superoxide dismutase-like activity[30]. Similar scenarios were observed in peroxidase-like nanozymes. A more effective approach for comparing these activities involves assessing the kinetics of catalytic reactions. Typically, controlling the pH, temperature and substrate concentration is necessary to measure these kinetics accurately. For instance, precise determination of the V_{max} value aids in comparing activities, investigating mechanisms and enhancing nanozyme activities. Presently, a standardized assay is employed to ascertain the V_{max} value of peroxidase-like nanozymes through a single fitting using the Michaelis–Menten equation. However, the genuine V_{max} value cannot be reliably confirmed using this method, owing to the constraint of a fixed substrate concentration. To address this limitation, a double-fitting method is introduced to establish the intrinsic V_{max} value by employing an additional Michaelis–Menten fitting[162]. Except for optimizing the fitting process, using the standard method that has been widely tested is another way to help researchers achieve reproducibility[27].

In vitro evaluation and diagnostics. In vitro evaluation, encompassing both cellular and bacterial assessments, necessitates adherence to established methodologies to ensure reproducibility of results. Diagnostics should precisely follow the recommended protocols and guidelines outlined by the assay manufacturer. This includes proper sample collection, handling and processing to obtain accurate and reliable diagnostic results. Factors such as temperature, humidity and sterility are necessary for the accuracy and reproducibility of evaluation.

In vivo therapy. Reproducibility in therapy refers to the ability of nanozymes to consistently deliver the intended therapeutics in different settings and across various subjects. Reproducibility relies on adhering strictly to standardized protocols related to the treatment of specific diseases. This encompasses ensuring that nanozymes are administered following well-defined procedures and dosages recommended for the targeted diseases. Maintaining strict quality control measures and accurately reporting methodologies, results and any deviations during experiments are also essential.

Data reporting

Ensuring reproducibility in nanozyme-based nanohealthcare demands particular attention to synthesis methods, standardized assessment methods and adherence to established protocols in both in vitro and in vivo settings. Transparent reporting and information sharing can enhance research reliability and replicability. Typically, the entire process is archived in electronic or laboratory note formats, allowing

global researchers to follow along. Details on nanozyme composition, structure, design strategies and safety should be well documented in academic publications. However, in the development of nanozyme-based nanohealthcare, established standards for storing data sets and other critical information are lacking. The absence of standardized procedures has hindered comparisons between different nanozymes. Uploading protocols and relevant data sets to cloud-based repositories (for example, GitHub) or specialized databases such as the nanozymes database could enhance transparency and accessibility, fostering more comprehensive analysis and validation. Moreover, to better construct the database, it is essential to provide not only the fundamental material characterization, enzymatic characteristics and biosafety information pertaining to a nanozyme but also crucial details such as storage methods, shelf life and other pertinent information facilitating its biomedical applications.

Limitations and optimizations

Design of nanozymes

The drug discovery process involves a trial-and-error approach, screening through compounds to identify lead compounds that can interact with biotargets such as proteins and nucleic acids[163]. Following high-throughput in vitro screening, the lead compounds progress as a clinical candidate[164]. Advancements in computing have popularized structure-based drug design[165,166]. This methodology also provides guidance for the development of therapeutic enzymes. The evolutionary pathway involves intricate selection for mutants, driving the direction of progress in this field[167]. For nanozymes, the overall pathways for nanohealthcare closely resemble those of drugs and therapeutic enzymes. However, several constraints remain.

Current approaches for the design of nanozymes may not be applicable to all nanozymes, owing to a lack of characterizing technique. The lack of tools for in situ monitoring of electronic structure and the interactions between substances and active sites poses significant challenges. Although numerous endeavours have been initiated to bridge these gaps, there remains much ground to cover.

There is still a vast amount of unexplored enzymatic activities. The current method to address this challenge involves emulating the structure of enzymes[168]. Furthermore, certain transformations involve multistep reactions that include electron transfer and the formation of intermediates. Accomplishing these steps solely with nanozymes is difficult; similar to enzymes, nanozymes might require co-factors to aid in these complex processes.

Targeting mechanisms for nanozymes

The precise targeting mechanisms of nanozymes are unknown. The lack of a clear understanding of how nanozymes precisely locate and interact with diseased sites or specific biomolecules hampers their effective utilization in therapies. The current targeting mechanisms of nanozymes are similar to those of nanomedicine where both active and passive targeting methods are commonly employed. In passive targeting, the enhanced permeability and retention effect exploits the inherent characteristics of the body's physiology to direct nanoparticles towards diseased areas[169]. However, the efficacy of this process must be re-evaluated, owing to the variable and often unpredictable results in animal experiments. Although progress has been made in uncovering the actual mechanisms behind the transportation of nanoparticles[170,171], the current understanding falls short in facilitating the transition from the design to practical application of nanozymes. There is a need to clarify the targeting mechanisms for further advancement.

Glossary

Activity descriptors
Parameters that can govern the activity of a catalyst.

Catalase
An enzyme that can convert H_2O_2 into water and oxygen.

Enzyme replacement therapy
A medical intervention in which a deficient or malfunctioning enzyme is supplemented or replaced with a synthetic or modified form of the enzyme to restore physiological functions and alleviate the symptoms associated with enzyme deficiencies or genetic disorders.

Hydrolase
An enzyme that can catalyse the hydrolysis of various chemical bonds through the addition of water molecules.

Interference study
A study focusing on assessing the impact of interfering substances on the accuracy of analysis.

Oxidase
An enzyme that can catalyse the transfer of electrons from a donor molecule to molecular oxygen.

Peroxidase
An enzyme that can catalyse reduction of H_2O_2 and other organic peroxides using an electron donor.

Self-cascade reactions
A series of consecutive reactions where the product of one enzymatic step becomes the substrate for the subsequent step, resulting in an efficient and self-sustaining cascade of reactions.

Superoxide dismutase
An enzyme that can catalyse the dismutation of superoxide radicals ($^{\bullet}O_2^-$) into oxygen and H_2O_2.

Biological effects of nanozymes

In a biological environment, nanozymes interact with various biomolecules, forming protein corona around their surface, which can alter the surface characteristics and functions of the nanozymes. In some cases, the protein corona can modify the properties of the nanozymes in beneficial ways. For example, the corona can provide a stealth effect, reducing recognition by the immune system to prolong the circulation time in the body. Additionally, the corona might enhance stability or modify the interaction of the nanozymes with their intended targets, potentially improving their overall performance[172]. Understanding and controlling the formation of the corona and its subsequent influence on the behaviour of the nanozymes is important to optimize their therapeutic efficacy.

To determine the therapeutic mechanisms and address potential biosafety concerns of nanozymes, the impact of nanozymes on biological barriers also needs to be understood. Receptor-mediated transcellular transport is a common approach for crossing the blood–brain barrier, where nanozymes are often modified with functional proteins or molecules[173]. However, variations in the chirality of molecules could result in different effects[174]. Moreover, certain nanozymes interacting with the body's systems might trigger circulatory inflammatory responses, potentially compromising the integrity of the blood–brain barrier and other important barriers[175].

Biodegradation of nanozymes refers to their breakdown into smaller components that can be easily eliminated from the body. The metabolism and potential biodegradability of nanozymes within the body can influence their safety, long-term effects and impact on the

body's homeostasis. The decomposition and clearance of nanozymes from the body is influenced by their composition, size, shape and surface properties. Some nanozymes may be metabolized and excreted through the kidneys, whereas other nanozymes might accumulate in other organs or tissues. Certain nanozymes may release ions during degradation, which can trigger an immune response. For example, Mn^{2+} ions possess immunostimulating capabilities that could be harnessed for disease treatment[176]. On the other hand, the biotransformation of materials such as molybdenum disulfide can result in the incorporation of molybdenum ions into molybdenum-dependent enzymes, particularly in the liver[177]. Similar to monitoring drug treatments, assessing the signals from nanozymes or their degradation products is essential in understanding their potential adverse effects. Developing a range of methodologies to assess both short-term and long-term toxicity is imperative. Vesicle-mediated secretion and organelle metabolism should be considered when exploring the impact of nanozymes in organ clearance to determine nanozyme biosafety[178].

Outlook

In the coming years, there are major challenges and opportunities that the research community must face. This section delves into the perspectives on nanozymes in nanohealthcare.

Rational design of nanozymes

The recent progress in nanozyme design, although promising, has not fully met the diverse needs of the field, which encompasses a wide array of enzyme-like activities. A promising avenue is to draw inspiration from enzymes, leveraging the lessons about the unique active sites and microenvironments that contribute to the superior activities of their biological counterparts. Several advancements have validated the potential of this tactic[168,179,180]. However, there is still a considerable journey ahead to fully harness the expansive possibilities of nanozymes. The distinctive features of enzymes, such as their intricate active sites and precisely tuned microenvironments, provide a rich source of inspiration for nanozyme design. Mimicking these facets introduces perspectives for optimization of nanozymes, potentially leading to enhanced catalytic activities and substrate specificities for diagnostics of specific disease biomarkers.

Artificial intelligence, through algorithms, has proven to be a powerful tool in materials science and bioinformatics[181–184]. Researchers can develop human–machine interaction-based strategies for the rational design of nanozymes using machine learning. This involves the use of algorithms to analyse vast data sets, identify patterns and predict optimal nanozyme structures for specific applications. This paradigm affords the opportunity to establish a universal artificial intelligence-assisted methodology for rational design of nanozymes.

Nanozyme-involved metabolism processes

Living systems might have incorporated catalytic elements from the environment, such as nanozymes, into their biological processes after the emergence of the first life forms[40,185,186]. This hypothesis suggests that nanozymes may contribute to the formation and development of essential biomolecules, eventually integrating into the biochemistry of living organisms. Exploring the role of nanozymes in the context of life evolution, particularly in essential metabolism processes (for example, the Krebs cycle)[187,188], is important to understand imperative metabolic reactions that serve as a linchpin for unravelling the mysteries behind metabolism-related conditions, including tumours and ageing. By delving into the evolutionary route of these crucial reactions, researchers can gain valuable knowledge to guide future evolutionary pathways, potentially offering perspectives on preventing or treating metabolic disorders.

Moreover, recognizing the potential involvement of nanozymes in metabolic reactions gives opportunities to create innovative tools for nanohealthcare. One such promising avenue involves combining nanozymes with organ-on-chip systems, which consist of engineered microfluidic chips mimicking the physiological environment of organs[189]. This hybrid approach could yield a simple yet potent disease model suitable for drug screening or cytotoxicity evaluation. By replicating the microenvironment of organs and incorporating nanozymes to mimic catalytic activities, these hybrid models provide a realistic platform for studying diseases. This approach is valuable for understanding complex metabolic disorders and their interplay with nanozyme-catalysed reactions. The hybrid models have the potential to transform our understanding of diseases, offering insights into the intricate interactions between nanozymes and metabolic pathways. This, in turn, can facilitate the development of new approaches in healthcare, ranging from innovative treatments to advanced diagnostics.

How do nanozymes function?

The promise of nanozymes in healthcare lies in their ability to interact with ROS through scavenging or generation. However, to translate these promises into therapeutic breakthroughs, the intricate mechanisms governing nanozyme treatments need to be further explored. Unravelling the detailed workings of nanozymes will offer comprehensive insights into the factors influencing their efficacy. Determining how nanozymes interact with biological systems, which cellular components are affected and the ensuing outcomes can aid in optimizing nanozyme-based treatments, ensuring both effectiveness and safety. Similar to small-molecule drugs, understanding the mechanisms of nanozyme treatments is essential to avoid and mitigate potential toxicities. This involves not only comprehending the immediate catalytic effects but also predicting and addressing any secondary effects that may arise. The aim is to tailor nanozyme treatments for maximum therapeutic benefit while minimizing adverse reactions.

Rodents are often used as primary animal models for in vivo studies of nanozymes. However, some studies have chosen dogs and monkeys to better mimic human disease conditions[98,127], facilitating future translational studies. By analysing the expression of proteins or genes and cell phenotypes, researchers can delve into the biological mechanisms of nanozymes, paving the way for future clinical applications. This kind of exploration will advance systems biology, uncovering potential disease target sites[190]. Meanwhile, systems biology approaches can provide insights into the complex biological processes underlying diseases, thereby guiding the design and optimization of nanozyme-based theranostics for improved efficacy and specificity. The integration of systems biology and nanozymes is also driving the exploration of emerging research directions, such as synthetic biology and personalized medicine. Future applications of nanozymes, especially in clinical use, should include treating diseases by synergizing with the body's systems (for example, the immune system)[191], as well as contributing to disease screening, such as liquid biopsy[192].

Standards and database of nanozymes

It is crucial to establish a standardized method for evaluating nanozyme activities; a standardized evaluation method will provide common

ground for researching different nanozymes. With diverse nanozymes emerging, ranging from various materials to structural designs, having a consistent benchmark will ensure that their performances can be objectively compared. A standardized evaluation framework will also allow clear and reproducible experiments to be established. Consistency in experimental protocols will enable studies to be replicated across different laboratories, reinforcing the reliability of results. This enhances the credibility of findings and accelerates the validation process for nanozyme-based theranostics. In addition, a standardized evaluation approach should foster collaboration and information exchange within the scientific community. This collaboration is vital for collective problem-solving, pooling knowledge and expertise to address challenges and innovate solutions in nanozyme-based theranostics.

Data sharing is beneficial for those entering the field, providing a comprehensive understanding of past experiments, outcomes and methodologies. The integration of artificial intelligence assistance on such a platform amplifies its utility. Artificial intelligence can screen through vast data sets, identifying patterns, correlations and potential areas for innovation. Through artificial intelligence, the process of designing nanozymes and devising novel applications can be streamlined to accelerate the research and development cycle.

In conclusion, although nanozymes hold potential for revolutionizing healthcare, acknowledging and addressing the current limitations and challenges is essential. By fostering collaborative efforts, the field of nanozymes for nanohealthcare can be propelled forwards, unlocking new possibilities for diagnosis, treatment and overall healthcare improvement.

Published online: 30 May 2024

References

1. Dulchavsky, M. et al. Directed evolution unlocks oxygen reactivity for a nicotine-degrading flavoenzyme. *Nat. Chem. Biol.* **19**, 1406–1414 (2023).
2. Pisal, D. S., Kosloski, M. P. & Balu-Iyer, S. V. Delivery of therapeutic proteins. *J. Pharm. Sci.* **99**, 2557–2575 (2010).
3. Kim, B. Y. S., Rutka, J. T. & Chan, W. C. W. Nanomedicine. *N. Engl. J. Med.* **363**, 2434–2443 (2010).
4. Torchilin, V. P. Recent advances with liposomes as pharmaceutical carriers. *Nat. Rev. Drug. Discov.* **4**, 145–160 (2005).
5. Thorek, D. L. J., Chen, A., Czupryna, J. & Tsourkas, A. Superparamagnetic iron oxide nanoparticle probes for molecular imaging. *Ann. Biomed. Eng.* **34**, 23–38 (2006).
6. Sperling, R. A., Rivera Gil, P., Zhang, F., Zanella, M. & Parak, W. J. Biological applications of gold nanoparticles. *Chem. Soc. Rev.* **37**, 1896–1908 (2008).
7. Dugan, L. L., Gabrielsen, J. K., Yu, S. P., Lin, T. S. & Choi, D. W. Buckminsterfullerenol free radical scavengers reduce excitotoxic and apoptotic death of cultured cortical neurons. *Neurobiol. Dis.* **3**, 129–135 (1996).
8. Pasquato, L., Rancan, F., Scrimin, P., Mancin, F. & Frigeri, C. *N*-Methylimidazole-functionalized gold nanoparticles as catalysts for cleavage of a carboxylic acid ester. *Chem. Commun.* **2000**, 2253–2254 (2000).
9. Comotti, M., Della Pina, C., Matarrese, R. & Rossi, M. The catalytic activity of "naked" gold particles. *Angew. Chem. Int. Ed.* **43**, 5812–5815 (2004).
10. Chen, J. P., Patil, S., Seal, S. & McGinnis, J. F. Rare earth nanoparticles prevent retinal degeneration induced by intracellular peroxides. *Nat. Nanotechnol.* **1**, 142–150 (2006).
11. Dugan, L. L. et al. Carboxyfullerenes as neuroprotective agents. *Proc. Natl Acad. Sci. USA* **94**, 9434–9439 (1997).
This paper presents C_{60} derivatives as neuroprotective drugs that exhibit anti-oxidant ability in biological systems.
12. Wei, H. & Wang, E. K. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. *Chem. Soc. Rev.* **42**, 6060–6093 (2013).
This review (and the cited articles within) describes development of nanozymes and gives a definition for nanozymes.
13. Wu, J. J. X. et al. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem. Soc. Rev.* **48**, 1004–1076 (2019).
14. Wei, H. et al. Nanozymes: a clear definition with fuzzy edges. *Nano Today* **40**, 101269 (2021).
This opinion provides an insightful viewpoint, elucidates crucial issues and encourages more profound contemplation for nanozyme development.
15. Huang, Y. Y., Ren, J. S. & Qu, X. G. Nanozymes: classification, catalytic mechanisms, activity regulation, and applications. *Chem. Rev.* **119**, 4357–4412 (2019).
16. Gao, L. Z. et al. Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat. Nanotechnol.* **2**, 577–583 (2007).
This paper presents the intrinsic peroxidase-mimic activity of Fe_3O_4 and starts a trend in nanozyme research.
17. Xi, Z. et al. Strain effect in palladium nanostructures as nanozymes. *Nano Lett.* **20**, 272–277 (2020).
18. Wu, H. A. et al. Enhanced tumor synergistic therapy by injectable magnetic hydrogel mediated generation of hyperthermia and highly toxic reactive oxygen species. *ACS Nano* **13**, 14013–14023 (2019).
19. Liu, Y. F. et al. Light-responsive metal–organic framework as an oxidase mimic for cellular glutathione detection. *Anal. Chem.* **91**, 8170–8175 (2019).
20. Tian, L. et al. Planar intercalated copper (II) complex molecule as small molecule enzyme mimic combined with Fe_3O_4 nanozyme for bienzyme synergistic catalysis applied to the microRNA biosensor. *Biosens. Bioelectron.* **110**, 110–117 (2018).
21. Cheng, H. J. et al. Integrated nanozymes with nanoscale proximity for in vivo neurochemical monitoring in living brains. *Anal. Chem.* **88**, 5489–5497 (2016).
22. Lin, A. Q. et al. Self-cascade uricase/catalase mimics alleviate acute gout. *Nano Lett.* **22**, 508–516 (2022).
23. Wang, Q. et al. A valence-engineered self-cascading antioxidant nanozyme for the therapy of inflammatory bowel disease. *Angew. Chem. Int. Ed.* **61**, e202201101 (2022).
24. Wang, Z. R., Zhang, R. F., Yan, X. Y. & Fan, K. L. Structure and activity of nanozymes: inspirations for design of nanozymes. *Mater. Today* **41**, 81–119 (2020).
25. Liu, B. W. & Liu, J. W. Surface modification of nanozymes. *Nano Res.* **10**, 1125–1148 (2017).
26. Loynachan, C. N. et al. Renal clearable catalytic gold nanoclusters for in vivo disease monitoring. *Nat. Nanotechnol.* **14**, 883–890 (2019).
This paper presents a renal clearable ultra-small nanozyme for in vivo tumour monitoring.
27. Jiang, B. et al. Standardized assays for determining the catalytic activity and kinetics of peroxidase-like nanozymes. *Nat. Protoc.* **13**, 1506–1520 (2018).
This protocol demonstrates a standard workflow for measuring and defining the catalytic activity units and kinetics for peroxidase-like nanozymes.
28. Jiang, D. W. et al. Nanozyme: new horizons for responsive biomedical applications. *Chem. Soc. Rev.* **48**, 3683–3704 (2019).
29. Lin, A. et al. A dopamine-enabled universal assay for catalase and catalase-like nanozymes. *Anal. Chem.* **94**, 10636–10642 (2022).
30. Liu, Y. F. et al. In vitro measurement of superoxide dismutase-like nanozyme activity: a comparative study. *Analyst* **146**, 1872–1879 (2021).
31. Murphy, M. P. et al. Guidelines for measuring reactive oxygen species and oxidative damage in cells and in vivo. *Nat. Metab.* **4**, 651–662 (2022).
32. Apak, R. et al. Methods to evaluate the scavenging activity of antioxidants toward reactive oxygen and nitrogen species (IUPAC Technical Report). *Pure Appl. Chem.* **94**, 87–144 (2022).
33. Fan, H. Z. et al. Surface ligand engineering ruthenium nanozyme superior to horseradish peroxidase for enhanced immunoassay. *Adv. Mater.* **36**, 2300387 (2024).
34. Duan, D. M. et al. Nanozyme-strip for rapid local diagnosis of Ebola. *Biosens. Bioelectron.* **74**, 134–141 (2015).
35. Liu, D. et al. Nanozyme chemiluminescence paper test for rapid and sensitive detection of SARS-CoV-2 antigen. *Biosens. Bioelectron.* **173**, 112817 (2021).
36. Zhou, D. T. et al. Orally administered platinum nanomarkers for urinary monitoring of inflammatory bowel disease. *ACS Nano* **16**, 18503–18514 (2022).
37. Zhang, Y. et al. Biomimetic design of mitochondria-targeted hybrid nanozymes as superoxide scavengers. *Adv. Mater.* **33**, 2006570 (2021).
38. Fu, S. Y. et al. ROS-targeted depression therapy via BSA-incubated ceria nanoclusters. *Nano Lett.* **22**, 4519–4527 (2022).
39. Lin, A. Q., Liu, S. J. & Wei, H. Nanozymes for biomedical applications in orthopaedics. *Particuology* **76**, 32–45 (2023).
40. Wang, X. Y., Hu, Y. H. & Wei, H. Nanozymes in bionanotechnology: from sensing to therapeutics and beyond. *Inorg. Chem. Front.* **3**, 41–60 (2016).
41. Shamsabadi, A., Haghighi, T., Carvalho, S., Frenette, L. C. & Stevens, M. M. The nanozyme revolution: enhancing the performance of medical biosensing platforms. *Adv. Mater.* **36**, 2300184 (2024).
42. Wang, H., Wan, K. W. & Shi, X. H. Recent advances in nanozyme research. *Adv. Mater.* **31**, 1805368 (2019).
43. Ghosh, S., Roy, P., Karmodak, N., Jemmis, E. D. & Mugesh, G. Nanoisozymes: crystal-facet-dependent enzyme-mimetic activity of V_2O_5 nanomaterials. *Angew. Chem. Int. Ed.* **57**, 4510–4515 (2018).
44. Chen, M. et al. Facet engineering of nanoceria for enzyme-mimetic catalysis. *ACS Appl. Mater. Interfaces* **14**, 21989–21995 (2022).
45. Jain, A., Shin, Y. & Persson, K. A. Computational predictions of energy materials using density functional theory. *Nat. Rev. Mater.* **1**, 1–13 (2016).
46. Medford, A. J. et al. From the Sabatier principle to a predictive theory of transition-metal heterogeneous catalysis. *J. Catal.* **328**, 36–42 (2015).
47. Norskov, J. K., Abild-Pedersen, F., Studt, F. & Bligaard, T. Density functional theory in surface chemistry and catalysis. *Proc. Natl Acad. Sci. USA* **108**, 937–943 (2011).
48. Norskov, J. K., Bligaard, T., Rossmeisl, J. & Christensen, C. H. Towards the computational design of solid catalysts. *Nat. Chem.* **1**, 37–46 (2009).
This review describes computational approaches for the discovery and development of catalysts.

49. Wang, X. Y. et al. e_g occupancy as an effective descriptor for the catalytic activity of perovskite oxide-based peroxidase mimics. *Nat. Commun.* **10**, 704 (2019).
50. Wang, Q. et al. e_g occupancy as a predictive descriptor for spinel oxide nanozymes. *Nano Lett.* **22**, 10003–10009 (2022).
51. Shen, X. M., Wang, Z. Z., Gao, X. J. J. & Gao, X. F. Reaction mechanisms and kinetics of nanozymes: insights from theory and computation. *Adv. Mater.* **36**, 2211151 (2024).
52. Wang, Z. Z. et al. Accelerated discovery of superoxide-dismutase nanozymes via high-throughput computational screening. *Nat. Commun.* **12**, 6866 (2021).
53. Li, S. R. et al. Data-informed discovery of hydrolytic nanozymes. *Nat. Commun.* **13**, 827 (2022).
54. Wei, Y. H. et al. Prediction and design of nanozymes using explainable machine learning. *Adv. Mater.* **34**, 2201736 (2022).
This paper presents an attempt for nanozyme design by machine learning.
55. Zhuang, J. et al. Machine-learning-assisted nanozyme design: lessons from materials and engineered enzymes. *Adv. Mater.* **36**, 2210848 (2024).
56. Huang, P., Wang, C. R., Deng, H. Z., Zhou, Y. F. & Chen, X. Y. Surface engineering of nanoparticles toward cancer theranostics. *Acc. Chem. Res.* **56**, 1766–1779 (2023).
57. Zandieh, M. & Liu, J. W. Surface science of nanozymes and defining a nanozyme unit. *Langmuir* **38**, 3617–3622 (2022).
58. Tang, G., He, J., Liu, J., Yan, X. & Fan, K. Nanozyme for tumor therapy: surface modification matters. *Exploration* **1**, 75–89 (2021).
59. Singh, S. et al. A phosphate-dependent shift in redox state of cerium oxide nanoparticles and its effects on catalytic properties. *Biomaterials* **32**, 6745–6753 (2011).
60. Xu, C., Liu, Z., Wu, L., Ren, J. & Qu, X. Nucleoside triphosphates as promoters to enhance nanoceria enzyme-like activity and for single-nucleotide polymorphism typing. *Adv. Funct. Mater.* **24**, 1624–1630 (2013).
61. Asati, A., Santra, S., Kaittanis, C., Nath, S. & Perez, J. M. Oxidase-like activity of polymer-coated cerium oxide nanoparticles. *Angew. Chem. Int. Ed.* **48**, 2308–2312 (2009).
62. Liu, B. & Liu, J. Accelerating peroxidase mimicking nanozymes using DNA. *Nanoscale* **7**, 13831–13835 (2015).
63. He, S.-B. et al. A heparinase sensor based on a ternary system of Hg^{2+}–heparin–osmium nanoparticles. *Anal. Chem.* **92**, 1635–1642 (2020).
64. Li, R. et al. Zwitterionic sulfhydryl sulfobetaine stabilized platinum nanoparticles for enhanced dopamine detection and antitumor ability. *ACS Appl. Mater. Interfaces* **14**, 55201–55216 (2022).
65. Ouyang, Y. et al. Aptamer-modified Cu^{2+}-functionalized C-dots: versatile means to improve nanozyme activities—"aptananozymes". *J. Am. Chem. Soc.* **143**, 11510–11519 (2021).
66. Wu, X., Chen, T., Chen, Y. & Yang, G. Modified Ti_3C_2 nanosheets as peroxidase mimetics for use in colorimetric detection and immunoassays. *J. Mater. Chem. B* **8**, 2650–2659 (2020).
67. Zhao, L. et al. The DNA controllable peroxidase mimetic activity of MoS_2 nanosheets for constructing a robust colorimetric biosensor. *Nanoscale* **12**, 19420–19428 (2020).
68. Zhao, Y. et al. Fluoride-capped nanoceria as a highly efficient oxidase-mimicking nanozyme: inhibiting product adsorption and increasing oxygen vacancies. *Nanoscale* **11**, 17841–17850 (2019).
69. Liu, B., Huang, Z. & Liu, J. Boosting the oxidase mimicking activity of nanoceria by fluoride capping: rivaling protein enzymes and ultrasensitive F^- detection. *Nanoscale* **8**, 13562–13567 (2016).
70. Fan, K. et al. Optimization of Fe_3O_4 nanozyme activity via single amino acid modification mimicking an enzyme active site. *Chem. Commun.* **53**, 424–427 (2017).
71. Zhou, Y. et al. Mesoporous encapsulated chiral nanogold for use in enantioselective reactions. *Angew. Chem. Int. Ed.* **57**, 16791–16795 (2018).
72. Zhang, Z., Zhang, X., Liu, B. & Liu, J. Molecular imprinting on inorganic nanozymes for hundred-fold enzyme specificity. *J. Am. Chem. Soc.* **139**, 5412–5419 (2017).
This paper presents a strategy to improve specificity of nanozymes by molecular imprinting.
73. Jiang, B., Fang, L., Wu, K. M., Yan, X. Y. & Fan, K. L. Ferritins as natural and artificial nanozymes for theranostics. *Theranostics* **10**, 687–706 (2020).
74. Fan, K. et al. Magnetoferritin nanoparticles for targeting and visualizing tumour tissues. *Nat. Nanotechnol.* **7**, 459–464 (2012).
This paper presents a nanozyme-based visualization approach for tumour tissues.
75. Xu, J. Q. et al. Design of diselenide-bridged hyaluronic acid nano-antioxidant for efficient ROS scavenging to relieve colitis. *ACS Nano* **16**, 13037–13048 (2022).
76. Cheng, C. Q. et al. Multifunctional nanozyme hydrogel with mucosal healing activity for single-dose ulcerative colitis therapy. *Bioconjug. Chem.* **33**, 248–259 (2022).
77. Wu, H. Y., Jia, P. P., Zou, Y. & Jiang, J. Cascade targeting tumor mitochondria with CuS nanoparticles for enhanced photothermal therapy in the second near-infrared window. *Biomater. Sci.* **9**, 5209–5217 (2021).
78. Li, S. R., Zhang, Y. H., Wang, Q., Lin, A. Q. & Wei, H. Nanozyme-enabled analytical chemistry. *Anal. Chem.* **94**, 312–323 (2022).
79. Chenthamara, D. et al. Therapeutic efficacy of nanoparticles and routes of administration. *Biomater. Res.* **23**, 20 (2019).
80. Tabish, T. A., Crabtree, M. J., Townley, H. E., Winyard, P. G. & Lygate, C. A. Nitric oxide releasing nanomaterials for cardiovascular applications. *J. Am. Coll. Cardiol. Basic. Trans. Sci.* **23**, 2579–2601 (2023).
81. Zhu, B. J. et al. Introducing nanozymes: new horizons in periodontal and dental implant care. *ChemBioChem* **24**, e202200636 (2023).
82. Peng, F. F., Zhang, Y. & Gu, N. Size-dependent peroxidase-like catalytic activity of Fe_3O_4 nanoparticles. *Chin. Chem. Lett.* **19**, 730–733 (2008).
83. Singh, N., Savanur, M. A., Srivastava, S., D'Silva, P. & Mugesh, G. A redox modulatory Mn_3O_4 nanozyme with multi-enzyme activity provides efficient cytoprotection to human cells in a Parkinson's disease model. *Angew. Chem. Int. Ed.* **56**, 14267–14271 (2017).
84. Zhao, S. et al. An orally administered CeO_2@montmorillonite nanozyme targets inflammation for inflammatory bowel disease therapy. *Adv. Funct. Mater.* **30**, 2004692 (2020).
85. Ding, S. et al. Effect of phosphorus modulation in iron single-atom catalysts for peroxidase mimicking. *Adv. Mater.* **36**, 2209633 (2024).
86. Ai, Y. et al. Ultra-small high-entropy alloy nanoparticles: efficient nanozyme for enhancing tumor photothermal therapy. *Adv. Mater.* **35**, 2302335 (2023).
87. Gao, L. et al. Standard vocabulary for nanozyme. *China Terminol.* **22**, 21–24 (2020).
88. Zandieh, M. & Liu, J. Nanozyme catalytic turnover and self-limited reactions. *ACS Nano* **15**, 15645–15655 (2021).
89. Ji, S. et al. Matching the kinetics of natural enzymes with a single-atom iron nanozyme. *Nat. Catal.* **4**, 407–417 (2021).
90. Gabrielli, L., Prins, L. J., Rastrelli, F., Mancin, F. & Scrimin, P. Hydrolytic nanozymes. *Eur. J. Org. Chem.* **2020**, 5044–5055 (2020).
91. Zhu, Z. et al. Translating molecular recognition into a pressure signal to enable rapid, sensitive, and portable biomedical analysis. *Angew. Chem. Int. Ed.* **54**, 10448–10453 (2015).
92. Villamena, F. A. & Zweier, J. L. Detection of reactive oxygen and nitrogen species by EPR spin trapping. *Antioxid. Redox Sign.* **6**, 619–629 (2004).
93. Zhang, W. et al. Prussian blue nanoparticles as multienzyme mimetics and reactive oxygen species scavengers. *J. Am. Chem. Soc.* **138**, 5860–5865 (2016).
This paper presents a systematic study of the enzymic activities of Prussian blue.
94. Komkova, M. A., Ibragimova, O. A., Karyakina, E. E. & Karyakin, A. A. Catalytic pathway of nanozyme "artificial peroxidase" with 100-fold greater bimolecular rate constants compared to those of the enzyme. *J. Phys. Chem. Lett.* **12**, 171–176 (2021).
95. Wu, G. et al. Critical comparison of the superoxide dismutase-like activity of carbon antioxidant nanozymes by direct superoxide consumption kinetic measurements. *ACS Nano* **13**, 11203–11213 (2019).
96. Bull, C., Niederhoffer, E. C., Yoshida, T. & Fee, J. A. Kinetic studies of superoxide dismutases: properties of the manganese-containing protein from *Thermus thermophilus*. *J. Am. Chem. Soc.* **113**, 4069–4076 (1991).
97. Nel, A. E. et al. Understanding biophysicochemical interactions at the nano–bio interface. *Nat. Mater.* **8**, 543–557 (2009).
This review describes the basics of the nano–bio interface.
98. Cao, F. F. et al. Artificial-enzymes-armed probiotics for alleviating intestinal inflammation and microbiota dysbiosis. *Nat. Nanotechnol.* **18**, 617–627 (2023).
99. Ma, M. M. et al. Self-protecting biomimetic nanozyme for selective and synergistic clearance of peripheral amyloid-β in an Alzheimer's disease model. *J. Am. Chem. Soc.* **142**, 21702–21711 (2020).
100. Jana, D. et al. Ultrasmall alloy nanozyme for ultrasound- and near-infrared light-promoted tumor ablation. *ACS Nano* **15**, 7774–7782 (2021).
101. Liu, W. L. et al. Integrated cascade nanozymes with antisenescence activities for atherosclerosis therapy. *Angew. Chem. Int. Ed.* **62**, e202304465 (2023).
102. Liao, K. H., Lin, Y. S., Macosko, C. W. & Haynes, C. L. Cytotoxicity of graphene oxide and graphene in human erythrocytes and skin fibroblasts. *ACS Appl. Mater. Interfaces* **3**, 2607–2615 (2011).
103. Guan, Y. J. et al. Ceria/POMs hybrid nanoparticles as a mimicking metallopeptidase for treatment of neurotoxicity of amyloid-β peptide. *Biomaterials* **98**, 92–102 (2016).
104. Xu, B. L. et al. Immunomodulation-enhanced nanozyme-based tumor catalytic therapy. *Adv. Mater.* **32**, 2003563 (2020).
105. Fan, Y. H. et al. Immobilization of nano Cu-MOFs with polydopamine coating for adaptable gasotransmitter generation and copper ion delivery on cardiovascular stents. *Biomaterials* **204**, 36–45 (2019).
106. Zhang, X. Y. et al. A metal–organic-framework incorporated vascular graft for sustained nitric oxide generation and long-term vascular patency. *Chem. Eng. J.* **421**, 129577 (2021).
107. Yang, Z. L. et al. Bioclickable and mussel adhesive peptide mimics for engineering vascular stent surfaces. *Proc. Natl Acad. Sci. USA* **117**, 16127–16137 (2020).
108. Myers, J. A., Curtis, B. S. & Curtis, W. R. Improving accuracy of cell and chromophore concentration measurements using optical density. *BMC Biophys.* **6**, 4 (2013).
109. Chen, Z. W., Wang, Z. Z., Ren, J. S. & Qu, X. G. Enzyme mimicry for combating bacteria and biofilms. *Acc. Chem. Res.* **51**, 789–799 (2018).
110. Liu, Q. Y. et al. Nanozyme-cosmetic contact lenses for ocular surface disease prevention. *Adv. Mater.* **35**, 2305555 (2023).
111. Feng, L. S. et al. Enhancement of nanozyme permeation by endovascular interventional treatment to prevent vascular restenosis via macrophage polarization modulation. *Adv. Funct. Mater.* **30**, 2006581 (2020).
112. Liang, M. M. & Yan, X. Y. Nanozymes: from new concepts, mechanisms, and standards to applications. *Acc. Chem. Res.* **52**, 2190–2200 (2019).
113. Wei, G. et al. A probiotic nanozyme hydrogel regulates vaginal microenvironment for *Candida* vaginitis therapy. *Sci. Adv.* **9**, eadg094 (2023).
114. Liu, Y. F. et al. Integrated cascade nanozyme catalyzes in vivo ROS scavenging for anti-inflammatory therapy. *Sci. Adv.* **6**, eabb2695 (2020).
115. Jiang, D. W. et al. Nanomedicines for renal management: from imaging to treatment. *Acc. Chem. Res.* **53**, 1869–1880 (2020).

116. Xu, Q. et al. Hypoxia-responsive platinum supernanoparticles for urinary microfluidic monitoring of tumors. *Angew. Chem. Int. Ed.* **61**, e202114239 (2022).
117. Wei, H. & Wang, E. Fe_3O_4 magnetic nanoparticles as peroxidase mimetics and their applications in H_2O_2 and glucose detection. *Anal. Chem.* **80**, 2250–2254 (2008).
This paper presents a classical approach for glucose detection using nanozymes.
118. Lee, H., Shin, W., Kim, H. J. & Kim, J. Turn-on fluorescence sensing of oxygen with dendrimer-encapsulated platinum nanoparticles as tunable oxidase mimics for spatially resolved measurement of oxygen gradient in a human gut-on-a-chip. *Anal. Chem.* **93**, 16123–16132 (2021).
119. Hu, Y. H. et al. Surface-enhanced Raman scattering active gold nanoparticles with enzyme-mimicking activities for measuring glucose and lactate in living tissues. *ACS Nano* **11**, 5558–5566 (2017).
120. Wang, X. Y., Qin, L., Zhou, M., Lou, Z. P. & Wei, H. Nanozyme sensor arrays for detecting versatile analytes from small molecules to proteins and cells. *Anal. Chem.* **90**, 11696–11702 (2018).
121. Kim, M. I., Park, K. S. & Park, H. G. Ultrafast colorimetric detection of nucleic acids based on the inhibition of the oxidase activity of cerium oxide nanoparticles. *Chem. Commun.* **50**, 9577–9580 (2014).
122. Li, W., Zhi, X., Yang, J. J., Zhang, J. L. & Fu, Y. Colorimetric detection of cysteine and homocysteine based on an oligonucleotide-stabilized Pd nanozyme. *Anal. Methods* **8**, 5111–5116 (2016).
123. Wang, X. Y. et al. Platinum–nickel nanoparticles with enhanced oxidase-like activity for total antioxidant capacity bioassay. *Anal. Chem.* **95**, 5937–5945 (2023).
124. Xu, Y. et al. The Fe–N–C nanozyme with both accelerated and inhibited biocatalytic activities capable of accessing drug–drug interactions. *Angew. Chem. Int. Ed.* **59**, 14498–14503 (2020).
125. Xu, Y. C. et al. In-ear integrated sensor array for the continuous monitoring of brain activity and of lactate in sweat. *Nat. Biomed. Eng.* **7**, 1307–1320 (2023).
126. Tao, X. Q., Wang, X., Liu, B. W. & Liu, J. W. Conjugation of antibodies and aptamers on nanozymes for developing biosensors. *Biosens. Bioelectron.* **168**, 112537 (2020).
127. Dugan, L. L. et al. Carboxyfullerene neuroprotection postinjury in Parkinsonian nonhuman primates. *Ann. Neurol.* **76**, 393–402 (2014).
128. Kwon, H. J. et al. Ceria nanoparticle systems for selective scavenging of mitochondrial, intracellular, and extracellular reactive oxygen species in Parkinson's disease. *Angew. Chem. Int. Ed.* **57**, 9408–9412 (2018).
129. Ma, X. X. et al. Prussian blue nanozyme as a pyroptosis inhibitor alleviates neurodegeneration. *Adv. Mater.* **34**, 2106723 (2022).
130. Gao, N. et al. Polyoxometalate-based nanozyme: design of a multifunctional enzyme for multi-faceted treatment of Alzheimer's disease. *Nano Res.* **9**, 1079–1090 (2016).
131. Du, Z., Li, M., Ren, J. S. & Qu, X. G. Current strategies for modulating Aβ aggregation with multifunctional agents. *Acc. Chem. Res.* **54**, 2172–2184 (2021).
132. Hou, W. D. et al. Excavating bioactivities of nanozyme to remodel microenvironment for protecting chondrocytes and delaying osteoarthritis. *Bioact. Mater.* **6**, 2439–2451 (2021).
133. Chande, S. & Bergwitz, C. Role of phosphate sensing in bone and mineral metabolism. *Nat. Rev. Endocrinol.* **14**, 637–655 (2018).
134. Pinna, A. et al. Nanoceria provides antioxidant and osteogenic properties to mesoporous silica nanoparticles for osteoporosis treatment. *Acta Biomater.* **122**, 365–376 (2021).
135. Karakoti, A. S. et al. Rare earth oxides as nanoadditives in 3-D nanocomposite scaffolds for bone regeneration. *J. Mater. Chem.* **20**, 8912–8919 (2010).
136. Zhang, Y. H., Liu, W. L., Wang, X. Y., Liu, Y. F. & Wei, H. Nanozyme-enabled treatment of cardio- and cerebrovascular diseases. *Small* **19**, 2204809 (2023).
137. Tian, R. Z. et al. Se-containing MOF coated dual-Fe-atom nanozymes with multi-enzyme cascade activities protect against cerebral ischemic reperfusion injury. *Adv. Funct. Mater.* **32**, 2204025 (2022).
138. Das, S. et al. The induction of angiogenesis by cerium oxide nanoparticles through the modulation of oxygen in intracellular environments. *Biomaterials* **33**, 7746–7755 (2012).
139. Augustine, R. et al. Nanoceria can act as the cues for angiogenesis in tissue engineering scaffolds: toward next-generation in situ tissue engineering. *ACS Biomater. Sci. Eng.* **4**, 4338–4353 (2018).
140. Fedeli, S. et al. Nanomaterial-based bioorthogonal nanozymes for biological applications. *Chem. Soc. Rev.* **50**, 13467–13480 (2021).
This review describes nanozyme-based bio-orthogonal reactions for biological applications.
141. Zhao, Q. et al. Copper-based SURMOFs for nitric oxide generation: hemocompatibility, vascular cell growth, and tissue response. *ACS Appl. Mater. Interfaces* **11**, 7872–7883 (2019).
142. Meng, X. Q. et al. High-performance self-cascade pyrite nanozymes for apoptosis–ferroptosis synergistic tumor therapy. *ACS Nano* **15**, 5735–5751 (2021).
143. Wang, D. D. et al. Self-assembled single-site nanozyme for tumor-specific amplified cascade enzymatic therapy. *Angew. Chem. Int. Ed.* **60**, 3001–3007 (2021).
144. Yu, Z. Z., Lou, R. X., Pan, W., Li, N. & Tang, B. Nanoenzymes in disease diagnosis and therapy. *Chem. Commun.* **56**, 15513–15524 (2020).
145. Gong, F. et al. Ultrasmall oxygen-deficient bimetallic oxide $MnWO_x$ nanoparticles for depletion of endogenous GSH and enhanced sonodynamic cancer therapy. *Adv. Mater.* **31**, 1900730 (2019).
146. Liang, Q. et al. A metal-free nanozyme-activated prodrug strategy for targeted tumor catalytic therapy. *Nano Today* **35**, 100935 (2020).
147. Zhang, X. Z. et al. Degradable ZnS-supported bioorthogonal nanozymes with enhanced catalytic activity for intracellular activation of therapeutics. *J. Am. Chem. Soc.* **144**, 12893–12900 (2022).
148. Huang, C. C. et al. Hydrogen-bonded organic framework-based bioorthogonal catalysis prevents drug metabolic inactivation. *Nat. Catal.* **6**, 729–739 (2023).
149. Wang, Z. et al. A bimetallic metal–organic framework encapsulated with DNAzyme for intracellular drug synthesis and self-sufficient gene therapy. *Angew. Chem. Int. Ed.* **60**, 12431–12437 (2021).
150. You, Y. W. et al. DNA-based platform for efficient and precisely targeted bioorthogonal catalysis in living systems. *Nat. Commun.* **13**, 1459 (2022).
151. Makabenta, J. M. V. et al. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. *Nat. Rev. Microbiol.* **19**, 23–36 (2021).
152. Jin, L. L. et al. Microenvironment-activated nanozyme-armed bacteriophages efficiently combat bacterial infection. *Adv. Mater.* **35**, 2301349 (2023).
153. Lee, J. et al. Exploration of nanozymes in viral diagnosis and therapy. *Exploration* **2**, 20210086 (2022).
154. Oh, M. J. et al. Nanozyme-based robotics approach for targeting fungal infection. *Adv. Mater.* **36**, 2300320 (2024).
155. Gao, L. Z. et al. Nanocatalysts promote *Streptococcus mutans* biofilm matrix degradation and enhance bacterial killing to suppress dental caries in vivo. *Biomaterials* **101**, 272–284 (2016).
156. Naha, P. C. et al. Dextran-coated iron oxide nanoparticles as biomimetic catalysts for localized and pH-activated biofilm disruption. *ACS Nano* **13**, 4960–4971 (2019).
This work presents nanozyme-based therapeutics for dental infection.
157. Pan, J. Z. et al. A single-cell nanocoating of probiotics for enhanced amelioration of antibiotic-associated diarrhea. *Nat. Commun.* **13**, 2117 (2022).
158. Wei, G. et al. Nanozyme-enhanced probiotic spores regulate the intestinal microenvironment for targeted acute gastroenteritis therapy. *Nano Lett.* **24**, 2289–2298 (2024).
159. Fang, L. et al. Metastable iron sulfides Gram-dependently counteract resistant *Gardnerella vaginalis* for bacterial vaginosis treatment. *Adv. Sci.* **9**, 2104341 (2022).
160. Li, J. W. et al. Nanoprobiotics for remolding the pro-inflammatory microenvironment and microbiome in the treatment of colitis. *Nano Lett.* **23**, 8593–8601 (2023).
161. Guo, X. et al. Chiral nanoparticle-remodeled gut microbiota alleviates neurodegeneration via the gut–brain axis. *Nat. Aging* **3**, 1415–1429 (2023).
162. Wang, Y. T., Li, T. & Wei, H. Determination of the maximum velocity of a peroxidase-like nanozyme. *Anal. Chem.* **95**, 10105–10109 (2023).
163. Imming, P., Sinning, C. & Meyer, A. Drugs, their targets and the nature and number of drug targets. *Nat. Rev. Drug. Discov.* **5**, 821–834 (2006).
164. Capdeville, R., Buchdunger, E., Zimmermann, J. & Matter, A. Glivec (ST1571, Imatinib), a rationally developed, targeted anticancer drug. *Nat. Rev. Drug. Discov.* **1**, 493–502 (2002).
165. Jorgensen, W. L. The many roles of computation in drug discovery. *Science* **303**, 1813–1818 (2004).
166. Schneider, G. & Fechner, U. Computer-based design of drug-like molecules. *Nat. Rev. Drug. Discov.* **4**, 649–663 (2005).
167. Turner, N. J. Directed evolution drives the next generation of biocatalysts. *Nat. Chem. Biol.* **5**, 567–573 (2009).
168. Zhang, L., Wang, H. & Qu, X. G. Biosystem-inspired engineering of nanozymes for biomedical applications. *Adv. Mater.* **36**, 2211147 (2024).
169. Shi, J. J., Kantoff, P. W., Wooster, R. & Farokhzad, O. C. Cancer nanomedicine: progress, challenges and opportunities. *Nat. Rev. Cancer* **17**, 20–37 (2017).
170. Sindhwani, S. et al. The entry of nanoparticles into solid tumours. *Nat. Mater.* **19**, 566–575 (2020).
171. Nguyen, L. N. M. et al. The exit of nanoparticles from solid tumours. *Nat. Mater.* **22**, 1261–1272 (2023).
172. Ren, J. Y. et al. Chemical and biophysical signatures of the protein corona in nanomedicine. *J. Am. Chem. Soc.* **144**, 9184–9205 (2022).
173. Xu, R. et al. Nanozyme-based strategies for efficient theranostics of brain diseases. *Coordin. Chem. Rev.* **501**, 215519 (2024).
174. Jiang, W. et al. Chiral metal–organic frameworks incorporating nanozymes as neuroinflammation inhibitors for managing Parkinson's disease. *Nat. Commun.* **14**, 8137 (2023).
175. Cheng, W. et al. The domino effect in inhaled carbon black nanoparticles triggers blood–brain barrier disruption via altering circulatory inflammation. *Nano Today* **48**, 101721 (2023).
176. Lv, M. Z. et al. Manganese is critical for antitumor immune responses via cGAS–STING and improves the efficacy of clinical immunotherapy. *Cell Res.* **30**, 966–979 (2020).
177. Cao, M. J. et al. Molybdenum derived from nanomaterials incorporates into molybdenum enzymes and affects their activities in vivo. *Nat. Nanotechnol.* **16**, 708–716 (2021).
This paper presents the bioavailability and biotransformation of nanomaterial (such as MoS_2) and offers a guide for the biosafety study of nanozymes.
178. Gao, J. C. et al. Intracerebral fate of organic and inorganic nanoparticles is dependent on microglial extracellular vesicle function. *Nat. Nanotechnol.* **19**, 376–386 (2024).
179. Zhang, R. F., Yan, X. Y. & Fan, K. L. Nanozymes inspired by natural enzymes. *Acc. Mater. Res.* **2**, 534–547 (2021).
180. Wu, J. J. X. & Wei, H. Efficient design strategies for nanozymes. *Prog. Chem.* **33**, 42–51 (2021).
181. Wang, H. C. et al. Scientific discovery in the age of artificial intelligence. *Nature* **620**, 47–60 (2023).

182. White, A. D. The future of chemistry is language. *Nat. Rev. Chem.* **7**, 457–458 (2023).
183. Camacho, D. M., Collins, K. M., Powers, R. K., Costello, J. C. & Collins, J. J. Next-generation machine learning for biological networks. *Cell* **173**, 1581–1592 (2018).
184. Zheng, Z. et al. A GPT-4 reticular chemist for guiding MOF discovery. *Angew. Chem. Int. Ed.* **62**, e202311983 (2023).
185. Miller, S. L. A production of amino acids under possible primitive earth conditions. *Science* **117**, 528–529 (1953).
186. Huber, C. & Wächterschäuser, G. α-Hydroxy and α-amino acids under possible hadean, volcanic origin-of-life conditions. *Science* **314**, 630–632 (2006).
187. Muchowska, K. B., Varma, S. J. & Moran, J. Nonenzymatic metabolic reactions and life's origins. *Chem. Rev.* **120**, 7708–7744 (2020).
188. Lane, N. & Martin, W. F. The origin of membrane bioenergetics. *Cell* **151**, 1406–1416 (2012).
189. Leung, C. M. et al. A guide to the organ-on-a-chip. *Nat. Rev. Methods Primers* **2**, 33 (2022).
190. Kitano, H. Systems biology: a brief overview. *Science* **295**, 1662–1664 (2002).
191. Koo, S. et al. Ceria–vesicle nanohybrid therapeutic for modulation of innate and adaptive immunity in a collagen-induced arthritis model. *Nat. Nanotechnol.* **18**, 1502–1514 (2023).
192. Martin-Alonso, C. et al. Priming agents transiently reduce the clearance of cell-free DNA to improve liquid biopsies. *Science* **383**, eadf2341 (2024).
193. Qin, L., Hu, Y. & Wei, H. in *Nanozymology: Connecting Biology and Nanotechnology* Ch. 4 (ed. Yan, X. Y.) 79–101 (Springer Nature, 2020).
194. André, R. et al. V_2O_5 nanowires with an intrinsic peroxidase-like activity. *Adv. Funct. Mater.* **21**, 501–509 (2011).
195. Khan, N. A. & Jhung, S. H. Synthesis of metal–organic frameworks (MOFs) with microwave or ultrasound: rapid reaction, phase-selectivity, and size reduction. *Coordin. Chem. Rev.* **285**, 11–23 (2015).

Acknowledgements

This work was funded by the National Natural Science Foundation of China (22374071), the National Key R&D Program of China (2019YFA0709200 and 2021YFF1200700), Jiangsu Provincial Key R&D Program (BE2022836), the PAPD Program, State Key Laboratory of Analytical Chemistry for Life Science (5431ZZXM2306) and Fundamental Research Funds for the Central Universities (202200325, 021314380228 and 021314380195). The writing has been refined with the assistance of ChatGPT with GPT-4 from OpenAI.

Author contributions

Introduction (Y.Z. and H.W.); Experimentation (Y.Z., T.L. and X.W.); Results (Y.Z., T.L., Y.W. and H.W.); Applications (G.W., W.L., M.Z., Y.L. and H.W.); Reproducibility and data deposition (all authors); Limitations and optimizations (all authors); Outlook (all authors).

Competing interests

The authors declare no competing interests.

Additional information

Peer review information *Nature Reviews Methods Primers* thanks Xiaoyuan Chen, Kelong Fan and the other, anonymous, reviewer(s) for their contribution to the peer review of this work.

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