Environmental Science Nano

ROYAL SOCIETY OF CHEMISTRY

PERSPECTIVE

Cite this: *Environ. Sci.: Nano*, 2024, **11**, 3268

Metalloenzyme-inspired approach to the design and applications of phosphatase-mimetic nanozymes. Bridging the inorganic and organic worlds

Pavel Janoš [a] and Pavel Janoš Jr. [b]

Received 22nd February 2024,
Accepted 28th April 2024

DOI: 10.1039/d4en00144c

rsc.li/es-nano

Nanozymes were introduced approximately 15 years ago as inorganic materials capable of mimicking the catalytic abilities of natural (protein-based) enzymes. While the catalytic efficiency of nanozymes typically does not match that of enzymes, their research gains special attention due to their potential advantages over conventional enzymes, particularly their higher resistance to adverse conditions. This article focuses on the utilization of cerium oxide for the catalytic acceleration of non-redox reactions (*e.g.*, dephosphorylation). It elucidates certain analogies between the functioning of conventional enzymes (metalloenzymes) and the nanozymatic activity of ceria, and the distinctions in the mechanisms of action between the two catalyst types. The unique catalytic (enzymatic) ability of cerium oxide is predetermined by the fine interplay between surface reactivity (associated with surface defects) and structural integrity (simplicity and stability of the subsurface crystalline structure). Limitations associated with the less flexible nature of cerium oxide are discussed, together with strategies to overcome them, which are based on the new concept of dynamic active sites. Possible generalizations to other metal oxide-based nanozymes are briefly mentioned.

Environmental significance

Currently, nanomaterials have become an integral part of our daily lives. Despite their initial purpose, these materials, or their residual components, are introduced into the environment, where they can accumulate and engage in intricate mutual reactions and interactions with the surrounding ecosystem. Many naturally occurring or industrially produced nanomaterials exhibit remarkable catalytic activities. In some cases, these activities may be considered enzyme-mimicking. A comprehensive understanding of the interactions occurring at the interface between (predominantly inorganic) nanoparticles and complex biomolecules not only lays the foundations for new industrial branches but also holds the potential to significantly contribute to resolving fundamental questions in contemporary science, such as the origins of life on Earth.

1. Introduction

By definition (enc. Britannica), "an enzyme is a substance that acts as a catalyst in living organisms". Typically, enzymes are biomolecules that are able to accelerate dramatically the chemical reactions in biomedia with exceptional selectivity under relatively mild conditions, representing in many aspects the dream of chemical engineers searching for efficient catalysts utilizable in industry.

About fifteen years ago, the ability of magnetite (Fe_3O_4) nanoparticles to accelerate the oxidation of organic substances was interpreted as an enzyme-mimetic activity.[1] This article started the era of inorganic analogues of natural enzymes – nanozymes, *i.e.*, nanosized particles exhibiting enzyme-mimetic ability. It is worth mentioning, however, that inorganic (nano) particles were used to catalyse bio-reactions years before the mentioned article was published – note the "clusters" (freshly precipitated cerium hydroxide) used by Sumaoka *et al.*[2] to destroy a (highly resistant) molecule of 3′,5′-cyclic adenosine-monophosphate (cAMP). Most probably, even earlier examples could be traced back in the history of chemistry.

Although the term "nanozymes" was questioned recently,[3] hundreds of articles published annually use it in a similar sense as we will do in this article – for inorganic substances with an ability to mimic natural enzymes. Iron oxides still remain the most extensively examined nanozymes, followed by cerium oxide, but many other substances were tested for

[a] *University of Jan Evangelista Purkyne, Faculty of Environment, Pasteurova 3632/15, 400 96 Ústí nad Labem, Czech Republic. E-mail: pavel.janos@ujep.cz*
[b] *CNR – Istituto Officina dei Materiali (IOM), Via Bonomea 265, 34136 Trieste, Italy. E-mail: pjanos@sissa.it*

their enzyme-mimicking ability.[4–8] So far, more than 1200 nanozymes have been developed in more than 200 institutions involved in nanozyme research around the world, and more than 3000 papers have been published.[9]

Nanozymology as a branch of nanoscience (nanotechnology, nanomedicine) utilizes the basic principles of physics to explain the complex phenomena occurring at the edge of biosciences using the language of chemistry. Rapidly growing numbers of published papers and the amount of information exceeding the capacity of a single research group, together with the inherently multidisciplinary nature of the research, are the factors that emphasize the need for completely new strategies for the development and application of nanozymes, combining theoretical and computational approaches with a full range of sophisticated experimental methods. Chen *et al.*[9] characterized today's phase in nanozymology as a shift from computer driven to data driven research. In this process, methods of machine learning and artificial intelligence will certainly play an important role.

Regardless of the great efforts that have been made, there is still a large gap in the efficiency of nanozymes compared to natural enzymes.[10] Learning from nature as suggested by Zhang *et al.*[10] is certainly a good idea how to improve the efficiency of nanozymes. However, more in-depth understanding of the reaction mechanisms is necessary. In recent times, some progress has been made especially in the investigation of nanozymes imitating the redox properties of enzymes. As iron and cerium oxides contain cations with the ability to change their oxidation state, they were mostly used to mimic the enzymes from the oxidoreductase family.[11–13] However, cerium oxide as a multifunctional nanozyme[14] accelerates also some non-redox (hydrolytic) reactions governing the crucial processes in living organisms. In a recently published review, Wu *et al.*[15] presented some examples of nanozymes with phosphatase-like activity and their applications in various areas ranging from the destruction of toxic substances to sensing purposes. They concluded that an array of nanozymatic materials with dephosphorylating activity is narrower in comparison with redox active nanozymes.

In this article we focus on the unique ability of cerium oxide to catalyse non-redox (hydrolytic) reactions under biologically relevant conditions, (*i.e.* on the phosphatase-like activity of cerium oxide in the first place). Certain analogies between "conventional" enzymes (metalloenzymes) and nanozymes will be demonstrated. We will assess the attempts to exploit these analogies in the design and application of new nanozymes. Limitations arising from the rigid structure of (inorganic) nanozymes will be pointed out together with possibilities to circumvent these limitations.

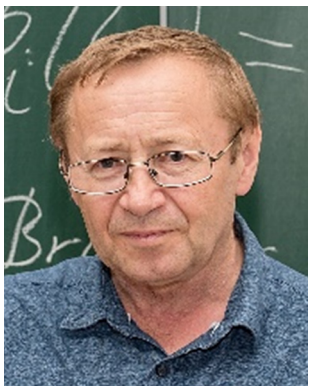
2. Inspiration sources for nanozyme development

2.1. From clusters to mixed-metal complexes

As mentioned, Sumaoka *et al.*[2] discovered the remarkable activity of cerium hydroxide precipitates (clusters). In parallel, they examined the application of various lanthanide complexes[16,17] and developed effective tools for DNA scission. It was shown that the best performance was exhibited by the complexes containing two different lanthanide cations, *e.g.* cerium and neodymium.[17] The activities of this group were reviewed recently by Komiyama.[18] The utilization of lanthanide cations and complexes has been a subject of extensive research and contributed significantly to understanding the mechanisms of the phosphoester bond cleavage.[19,20]

2.2. From reactive sorbents to nanozymes

At the turn of the century, Klabunde and his group at Kansas University found that nanosized MgO (ref. 21) and some other metal oxides (CaO, ZnO, TiO_2) are able not only to



Pavel Janoš

Pavel Janoš graduated from the University of Chemical Technology in Pardubice (1981) and Charles University in Prague (PhD, 1992) in analytical chemistry. Since 1983, he has been a researcher at the Research Institute of Inorganic Chemistry, Ústí nad Labem, involved in the chemistry of rare earths. Since 2002, he has been a professor/senior scientist at the University of J. E. Purkyně, Faculty of Environment, Ústí nad Labem, involved in the research of non-conventional sorbents and nanomaterials for environmental applications.

Pavel Janoš Jr.

Pavel Janoš, (Jr.) obtained his PhD in Biomolecular Chemistry from Masaryk University, Brno (2019) studying the reaction mechanism of glycosyltransferases, enzymes catalyzing the formation of glycosidic bonds. Since 2020, he has been a post-doctoral fellow at CNR - Istituto Officina dei Materiali (IOM), Trieste focusing on metal ion transporters, proteins transferring metal ions across membranes, before switching to studies of spliceosome and protein/RNA complexes in general. Currently, he is a researcher at CNR – Istituto Officina dei Materiali (IOM), Trieste pursuing the development of RNA- and/or protein/RNA-targeted therapeutics.

retain, on their surfaces, some dangerous compounds including highly toxic phosphate-based chemical warfare agents (CWAs), but also to convert them (to destroy) into non-toxic or less toxic degradation products.[22–25] These materials, under the common name of reactive sorbents, have been successfully commercialized and are still used by militaries and security forces around the world as an effective means of combating highly dangerous CWAs. In addition to those mentioned above, several other metal oxides including lanthanide oxides have been tested as reactive sorbents. Interestingly, with a rare exception,[26] cerium oxide was not considered as an effective reactive sorbent. Even in the mentioned report,[26] cerium oxide was classified as a poorly effective decontamination agent.

More recently, Štengl *et al.*[27–29] extended substantially the family of reactive sorbents and developed new, environmentally friendly synthetic procedures suitable for their large-scale production. Reactive sorbents were used to destroy organophosphate pesticides – substances structurally similar to the mentioned CWAs, *e.g.* parathion methyl or paraoxon methyl.[30] Cerium oxide was introduced by Janoš *et al.*[31–33] as a reactive sorbent capable of destroying both CWAs and organophosphate pesticides. Inspired by the pioneering work of Kuchma *et al.*,[34] some other environmentally relevant reactions of cerium oxide were examined,[35] including its ability to mimic natural enzymes. The methods of the preparation of cerium oxide are briefly summarized in the following section.

Preparation of cerium oxide

Cerium as the most abundant element of the lanthanide group is easily available. Due to the so called rare earth balance problem[36] it is produced in such quantities that markedly exceed the demand. Cerium oxide is the most important cerium compound with traditional large-scale applications in glass polishing and catalysis, and its applications in biosciences and medicine that are usually related to its nanocrystalline forms have been rapidly growing in recent times. Numerous synthetic methods were developed for these emerging applications of cerium oxide – see *e.g.* reviews.[37–43] For the purpose of this article, the synthetic methods can be divided into two groups: i) the precipitation/calcination method requires the preparation of an insoluble cerium-containing precursor (typically carbonate, oxalate or hydroxide), which is subsequently converted to cerium oxide by annealing at relatively high temperatures (500–600 °C) in the presence of oxygen. This method ("dry" or "high temperature") is suitable also for industrial applications, *e.g.* for the preparation of ceria-based polishing powders.[44,45] ii) The method of direct precipitation ("wet" or "low temperature") is derived from the more than one century old work of Dennis and Magee[46] and is still frequently used in many modifications mainly for the preparation of biocompatible forms of nanoceria. In this method, the aqueous solution of cerous salt is precipitated with the solution of ammonia (less often NaOH or KOH) at mild (*e.g.* ambient or even lower) temperature. Under extensive agitation (stirring, purging with decarbonized air) the sparingly soluble cerous hydroxide is converted to cerium oxide, which can be directly (without any additional treatment, such as drying) used for selected applications. Both synthetic procedures may be modified in numerous ways. For example, the precursor in the first procedure can be prepared by homogeneous precipitation,[33,47,48] using templates[49] or varying the conditions during precipitation.[50] Hydrothermal methods can be used to modify the properties of the precursor in the first synthetic route and also to modify the properties of cerium oxide in the second synthetic route; they are especially effective in the shape sensitive preparation of cerium oxide.[51,52] The methods of the cerium oxide preparation and conditions during its synthesis significantly affect most of its characteristics, such as morphology, surface area and surface chemistry, crystallinity, and many others. However, the relation between the synthetic route and nanozymatic activity is not straightforward. For example, cerium oxide prepared by the wet synthetic method at ambient temperature without any additional thermal treatment exhibited an almost identical dephosphorylating ability to cerium oxide prepared by thermal decomposition of cerium carbonate at 600 °C. This somewhat counter-intuitive finding is discussed in the main text.

3. Mechanisms of the phosphoester bond cleavage

Organophosphates, esters of the phosphoric (or thiophosphoric) acid, are a large group of diverse compounds differing, among others, in their susceptibility to undergo the hydrolytic cleavage of the phosphoester bonds (and subsequently in their persistence in the environment). Phosphomonoesters are usually energetically rich molecules, which may be easily destroyed liberating the terminal phosphate group (*e.g.* ATP). Phosphodiesters are very stable compounds with the phosphodiester bonds extremely resistant to hydrolytic cleavage. The phosphoester bonds in phosphotriesters are moderately strong. In summary, the reactivities of phosphates for hydrolytic cleavage follow usually the order:[53] pyrophosphates > phosphomonoesters > phosphotriesters ≫ phosphodiesters.

Phosphatases – enzymes effective in the cleavage of phosphoester bonds – often contain metal cations in their molecule; purple acid phosphatase (PAP) may serve as an example,[54] containing typically the Fe^{3+}/Fe^{2+} pair, in which Fe^{2+} may be replaced with some other divalent metal cations. More than one third of the known enzymes are classified as metalloenzymes;[55] all significant groups of enzymes, such as oxidoreductases, transferases or hydrolases, are involved here. In general, the role of metal cations consists typically in their ability to coordinate the central atom in the substrate and to activate the water molecule making it an effective nucleophilic agent. In PAP, the metal-ion-bound hydroxide is proposed to perform the nucleophilic attack.[56,57] Hence, the nucleophilic substitution is the mechanism responsible for the cleavage of the phosphoester bonds. Two modes of action of the metal ions in enzymatic reactions suggest that the presence of two metal cations in the metalloenzyme structure (active site) might be beneficial.[58] PAP catalyses typically the dephosphorylation of phosphomonoesters. Huang[59,60] demonstrated that iron oxide nanoparticles and even aged iron solutions exhibit phosphatase-like activity; however, a detailed mechanism of the dephosphorylating reactions was not given.[56,61]

Hence, we postulate that metal oxide-based nanozymes and some other materials (metalloorganic frameworks, MOFs) can be viewed as a certain kind of metalloenzymes. The analogy between nanozymes and metalloenzymes lies in their catalytic mechanism. Both types of catalysts use the same principles of catalysis, *e.g.*, a transition-state stabilization, and they both

exploit the concept of active sites/centres, where the substrate molecule binds and undergoes a chemical transformation.

The concept of active centres used in heterogeneous catalysis can be applied to enzyme-mimetic systems. Ideally, the active centre should[62]

– Stabilize (selectively) the transition state of the reaction it catalyses,

– Transform an intermolecular reaction into a (pseudo) intramolecular one by binding the reactants,

– Facilitate the proton transfer, stabilize charges, coordinate metal ions, and/or serve as a nucleophile and/or an electrophile,

– Modify the reaction microregion (hydrophilicity/hydrophobicity, pH) to be suitable for the given reaction.

According to the conventional Michaelis–Menten model, the enzymatically catalysed reaction is described by the following equation:[63]

$$E + S \Leftrightarrow ES \rightarrow EP \rightarrow E + P \quad (1)$$

The catalysed reaction, in which the reactant (substrate) is converted to the product, could be viewed as a cycle (see Fig. 1), into which the catalyst (enzyme) is introduced and is regenerated at the end of the cycle.[64] It should be noted that the scheme in Fig. 1 does not represent any particular reaction mechanism; the concept dividing the catalytic cycle into three parts allows us to discuss separately and (to some extent) independently the analogies and differences between enzymes and nanozymes for the distinct parts of the catalytically accelerated process. Before such a generalization, we will give some examples.

3.1. Hydrolytic cleavage of phosphotriesters as the model of the enzymatically and nanozymatically catalysed reaction

Phosphotriesters are toxic, artificially created derivatives of phosphoric acid. During the decades since their introduction into the environment (mainly as pesticides), some bacteria developed enzymes capable of destroying these substances – phosphotriesterases (PTEs), for example OPH from *Pseudomonas diminuta* or OpdA from *Agrobacterium radiobacter*.[65] PTEs, together with other enzymes with the ability to degrade the organophosphate compounds, attract considerable attention not only because of the urgent need to destroy great amounts of the banned pesticides, but as a means of combating chemical weapons (sarin, soman, and VX agent).

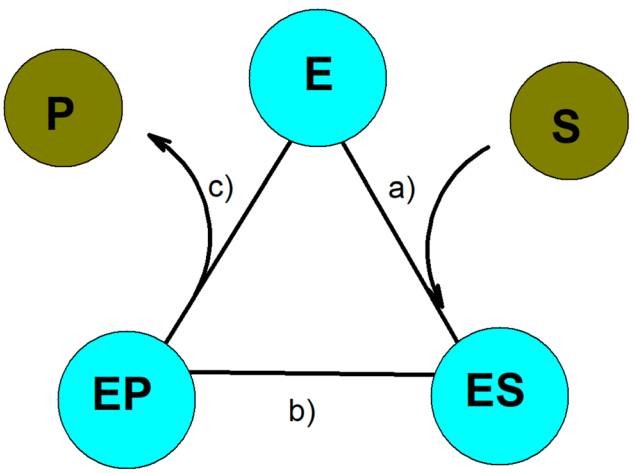


Fig. 1 Catalytic cycle. a) Interaction enzyme (nanozyme) with the substrate; b) chemical transformation; c) product liberation and regeneration of the active centre.

PTE isolated from *Pseudomonas diminuta* (OPH) was characterized by Aubert *et al.*[66] by a variety of spectroscopic and other techniques. It was used to destroy the organophosphate pesticide paraoxon and several of its structurally similar analogues with the goal of precisely describing the degradation mechanism. The binuclear metal centre with two zinc ions was identified as the catalytically active part of PTE. The destruction of paraoxon with PTE proceeds *via* a hydrolytic reaction, giving *p*-nitrophenol as a product. For a similar purpose, nanocrystalline cerium oxide was used, again with *p*-nitrophenol as a product.[67]

In PTEs, the phosphoester cleavage proceeds on active centres consisting of two metal cations bridged by hydroxide.[67] The metal cations, which may be identical, play different roles in the cleavage reaction. The cation at the β site polarizes the P–O bond in the phosphoester molecule by the interaction with phosphoryl oxygen, making the central P atom more susceptible to the nucleophilic attack. The cation at the α site with the hydroxide group, either the metal-bridging-OH or an α-bound water molecule (depending on the enzyme and/or substrate), serves as a nucleophile.[68–70]

The active centre on the cerium oxide surface consists of two cerium cations (advantageously in different oxidation states) and a hydroxyl group in a proper spatial arrangement. The Ce^{3+} cation corresponds to the α site in the binuclear metallozyme, and the Ce^{4+} cation corresponds to the β site. The phosphoester bonds are broken by the nucleophilic substitution mechanism in a similar way to that of metalloenzymes (Fig. 2). The concept of active centres is close to that of the vacancy/defect-engineered "hotspots" created by the chemical modification of cerium oxide[71] or by doping with a polyvalent metal cation.[72]

3.2. Dephosphorylation of cAMP

As stated above, the phosphodiester bonds are extremely resistant against the hydrolytic cleavage, which is quite understandable, considering the crucial role they play in ascertaining the stability of DNA and other important biomolecules. The phosphodiester bonds occur also in some small molecules, such as in cyclic adenosine monophosphate (cAMP). cAMP is a small molecule regulating (as a so called second messenger) many physiological processes both in plants and animals. In the absence of catalysts, the cAMP molecule is extremely resistant to the hydrolytic cleavage even at elevated temperature; Chin and Zou[73] estimated the half-

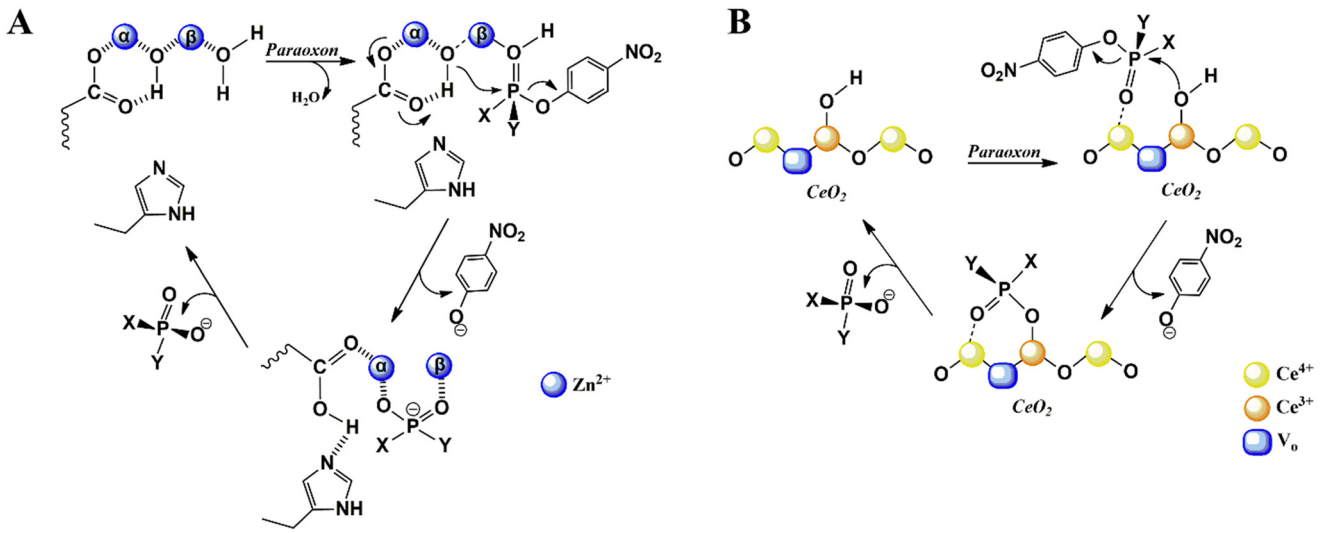


Fig. 2 (A) Degradation of paraoxon with bacterial PTE (simplified from ref. 66); (B) degradation of paraoxon on the surface of cerium oxide. This figure demonstrates clearly the different roles of α and β sites in binuclear metallozymes and analogous roles of Ce^{3+} and Ce^{4+} cations in cerium oxide. V_O represents oxygen vacancies.

life of uncatalyzed cAMP dephosphorylation to be a million years. Janoš *et al.*[74] demonstrated a remarkable enhancement of the reaction rate in the presence of cerium oxide; the half-lives for the total dephosphorylation (giving adenosine as a product) were in the range of several minutes to several hours. cAMP was recommended for testing the activity of the phosphodiesterase enzymes,[75] supposing that it may serve as a DNA surrogate. The cAMP stability was proven, among others, in tests imitating the conditions prevailing on Earth at the time of the origin of life.[76]

In living organisms, cAMP is hydrolysed by the phosphodiesterase enzyme (PDE) to give adenosine 5′-monophosphate (5′-AMP) as a product (Fig. 3A). PDE like PTE belongs to metalloenzymes with two metal cations in their active site. However, there are some differences between the two enzymes. In PTE, both metal cations are identical (Zn^{2+}), whereas PDE is a hetero-bimetallic protein containing different cations (Zn^{2+}, Mg^{2+}, Ca^{2+}).[77] Fig. 3C shows the overall structure of the PDE enzyme with different cations. The mechanism of the phosphodiester bond cleavage in cAMP was studied in detail by QM/MM simulation.[78] The pre-reaction complex of PDE with cAMP based on the aforementioned theoretical study is shown in Fig. 3D, highlighting the nucleophilic OH^- ion, the two catalytic cations (Zn^{2+}, Mg^{2+}) and amino acid side chains forming the catalytic centre of the enzyme.

It is assumed that nanozymatically catalysed dephosphorylation of cAMP proceeds by the S_N2 reaction mechanism. However, in the presence of cerium oxide, any possible intermediate (neither 5′-AMP nor 3′-AMP) was not detected, suggesting that the ring-opening reaction is followed immediately by the removal of the remaining phosphate group, giving adenosine as the final product.[74] A possible structure of the transition state is given in Fig. 3B.

4. Comparison of the efficiency of enzymes and nanozymes

A systematic comparison of nanozymes and natural enzymes in terms of their efficiency under comparable conditions can be rarely found in the literature. A critical comparison of nanozymes and enzymes for biosensing purposes given by Ashrafi *et al.*[80] contains some kinetic parameters (the K_m and K_{cat} constants of the Michaelis–Menten equation). However, only materials mimicking enzymes from the oxidoreductase family are involved here. The standardized procedure was developed to compare the activity of nanozymes with peroxidase-like ability.[81] According to this procedure, the activity of nanozymes is standardized against the activity of Fe_3O_4. Obviously, this procedure can be hardly adapted to other kinds of nanozymes.

Generally, the efficiency of catalysts is expressed using the terms such as the turnover number or turnover frequency, often used inconsistently in industrial catalysis and enzymology.[82] In this article, we use the term turnover frequency (TOF) calculated as the number of substrate molecules converted to the product by a single molecule of enzyme per second.[83] For natural enzymes, the TOF values vary in a wide range of few molecules to several million molecules per second. It should be noted that some phosphoesterases have the highest known enhancement rate, increasing the reaction rate as much as 10^{21} times over the uncatalyzed reaction.[83]

For nanozymes, the TOF values are reported less often; a possible reason is an unclear definition of the nanozyme unit,[84,85] which should replace the "molecule of enzyme" in the above definition. To give readers some perspective, we compare the degradation of the organophosphate pesticide paraoxon in the presence of cerium oxide[67] with the

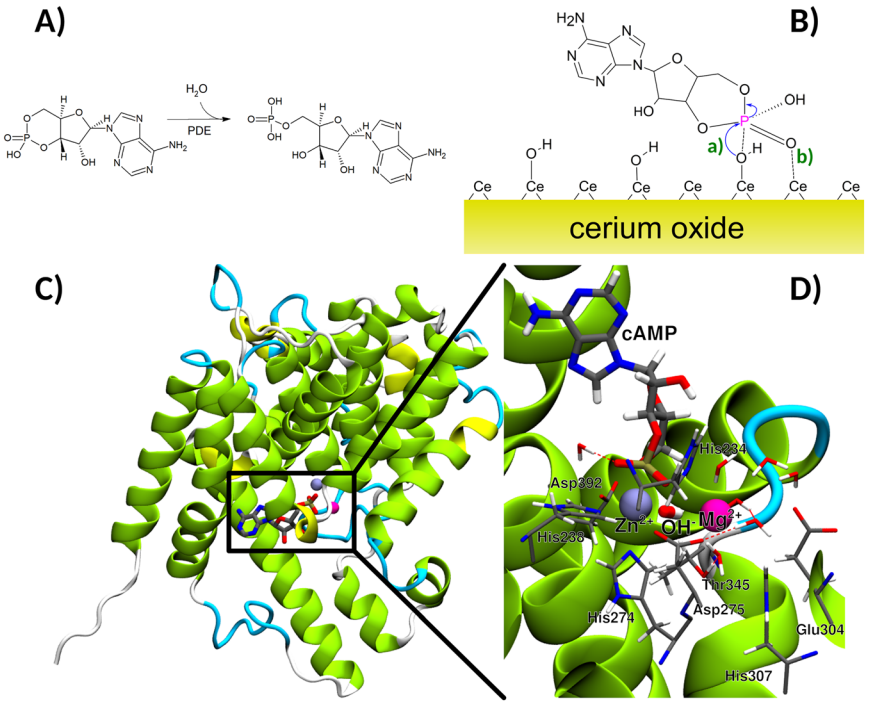


Fig. 3 A) Hydrolysis of cAMP by phosphodiesterase (PDE); B) schematic representation of the transition state in the ring-opening reaction of cAMP in the presence of cerium oxide. i) Coordination of the cerium cation by an oxygen atom and ii) nucleophilic attack of the OH group on the phosphorus atom;[67] C) structure of the PDE enzyme with bound AMP and the two catalytic metal ions (PDB ID: 1ROR).[79] The protein is displayed in new cartoon representation with α-helices, turns and coil regions shown in green, cyan and white, respectively. AMP is shown in licorice representation with atoms colored by element. The Zn^{2+} and Mg^{2+} catalytic metal ions are shown as purple and magenta spheres, respectively. D) Close-up of the PDE active site showing the pre-reaction complex modeled using Schrodinger's suite (2021) based on the information from ref. 78. The cAMP and the hydroxide ion, performing the nucleophilic attack in the reaction, are shown in thicker licorice representation. Protein residues and water molecules stabilizing the two metal ions and/or the cAMP's phosphate group are shown in thinner licorice representation with the hydrogen bonding interaction between them shown as red dashed lines.

enzymatically catalysed cleavage of the same pesticide by phosphotriesterase (PTE) from *Pseudomonas diminuta.*[66] As can be seen from kinetic dependencies and experimental conditions in ref. 67, the degradation of paraoxon proceeds almost completely within the time scale of several minutes in the presence of 50 mg cerium oxide. Considering CeO_2 as a nanozyme unit, we obtain the TOF values ranging from 10^{-7} to 10^{-5} s^{-1}.

Even lower TOFs were obtained for the dephosphorylation of cAMP in the presence of cerium oxide[74] (10^{-9} s^{-1}). For this reaction, the Michaelis–Menten equation can be used to fit the dependence of the dephosphorylating reaction rate on the initial concentration of cAMP (Fig. 4).

However, if we re-define the active centre as an assembly of the Ce^{3+} and Ce^{4+} cations and surface OH groups,[74] we can use the number of the surface OH groups (experimentally accessible) as an estimate of the nanozyme units. Then, TOFs in the order ranging from *ca.* 0.1 to 0.4 s^{-1} can be obtained. These values are still lower than the TOF value of the paraoxon hydrolysis by bacterial PTE (2300 s^{-1}), but the difference is not so dramatic and gives hope that with a suitable modification of either the nanozyme itself or the reaction conditions, it will be possible to achieve comparable activity of enzymes and nanozymes.

5. Can nanozymes compete with natural enzymes?

Enzymes are catalytically active biomacromolecules made of linear polypeptide/protein chains built from as much as 22 possible amino acids. The protein enzymes have been fine-tuned by nature for the catalysis of a wide range of reactions. This is possible thanks to the diverse "alphabet" of the 22 amino acids, from which proteins are constructed. The diversity of these building blocks enables proteins to adapt different global structures (folds)[86,87] with highly optimized local structures (active sites)[88] leading to very specialized biocatalysts both in terms of the reactions catalysed and the substrates being processed. The complexity of enzymes allows them to exploit the traditional "tricks" utilized in catalysis (stabilization of the transition state, destabilization of the ground state); their main advantage consists in their ability to arrange the substrate molecule and active site into such mutual positions where the respective reaction is most favoured.

Enzymes can stabilize the transition state of the reaction by, for instance, having a charge distribution in the active site that complements the electrostatic potential changes during the reaction,[89] or they can destabilize/disrupt the

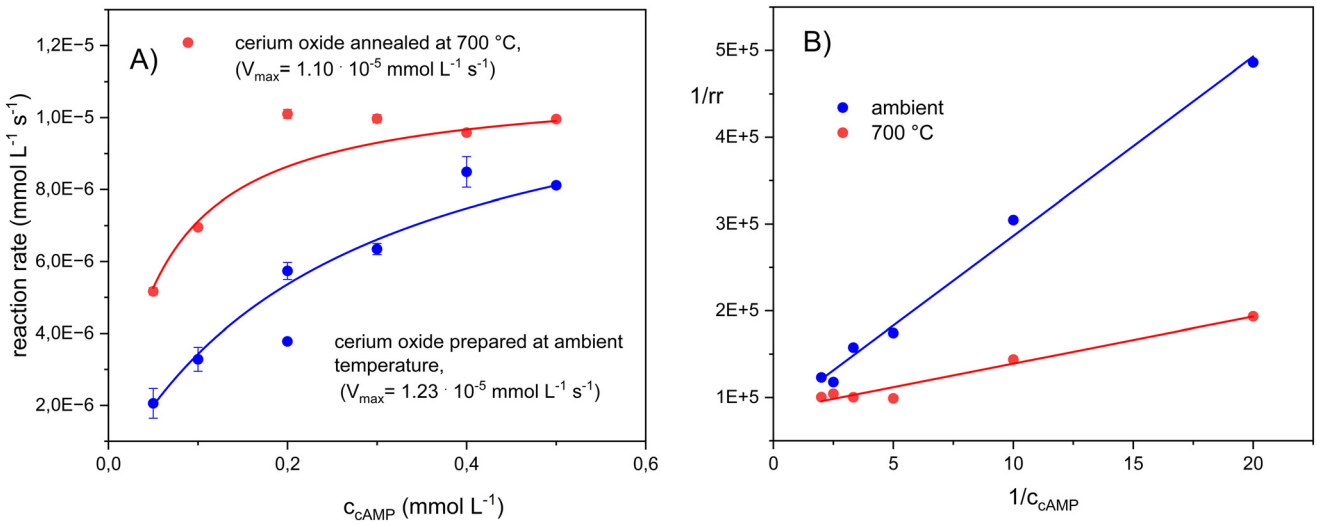


Fig. 4 A) Dependencies of the reaction rate on the initial concentration of substrate for the dephosphorylation of cAMP in the presence of different kinds of cerium oxide. Experimental data fitted to the Michaelis–Menten equation. B) Lineweaver–Burk plot used to treat the same experimental data. Experimental conditions: Concentration of cAMP ranged from 0.05 to 0.5 mmol L^{-1}, concentration of cerium oxide 12.5 mg mL^{-1}, TRIS buffer 0.01 mol L^{-1}, pH 7.043, temperature 25 ± 2 °C. Red points and curves – cerium oxide prepared from the carbonate precursor by calcination at *ca.* 700 °C, the blue points and curves – cerium oxide prepared by precipitation with ammonia and lyophilization. Data from: A. Alikberov, diploma thesis, University of J. E. Purkyně in Ústí nad Labem, 2024.

ground state of the reaction thus promoting easier transition into the transition state.[90]

The key to the function of enzymes is the nature of their active sites, which is (usually) a surface-exposed pocket or groove that facilitates the binding of a specific substrate and the catalysis of a specific reaction. The active part of the enzyme typically consists of the binding site and the catalytic site.[91] Catalytic sites are often similar for the enzyme group catalysing the same reaction with different substrates. As an example, PTEs from *Pseudomonas diminuta* (OPH) and *Agrobacterium radiobacter* (OpdA) share the same catalytic site only differing in three amino acids in the binding site, which drives their different substrate specificities.[65] In this part of the catalytic cycle (Fig. 1), we can find some similarity between nanozymes and natural enzymes.

As shown above, the dephosphorylating reactions proceed *via* the nucleophilic substitution reaction mechanism; these reactions are favoured in nonpolar or aprotic solvents. Natural enzymes are able to modify the reaction microregion and provide a different chemical environment (pH, hydrophobicity, *etc.*) more favourable to the catalysed reaction[92–94] compared to the solvent (reaction medium). Cerium oxide, although hydrophilic by nature, exhibits hydrophobic behaviour when immersed in water. Molecular dynamics simulation[95] demonstrated that the layer of adsorbed water molecules in contact with the surface of cerium oxide creates a hydrophobic interface, which may alter the conditions for the surface reactions. Some other studies confirmed a possibility to modify the hydrophobicity/hydrophilicity of the cerium oxide surface.[95,96] It was shown by molecular dynamic simulation that organophosphates such as thiamine pyrophosphate or β-nicotinamide adenine dinucleotide (NAD) interact with the surface of cerium oxide; the attractive interactions predominate over the competitive ceria–water interactions,[97] which makes the nanoceria-catalysed dephosphorylation reactions possible.

The binding site is what determines the substrate specificity of enzymes.[98] The binding of the substrate is driven by non-covalent interactions: hydrophobic interactions, electrostatic interactions, hydrogen bonding and others.[99,100] In this part of the catalytic cycle, the (purely inorganic) nanozymes may hardly compete with natural enzymes. For the given compound, such as cerium oxide, there are only a limited number of ways how to improve the accessibility of the catalytic site or even to optimize the geometry of the pre-reaction complex. To some extent, the difference in the reactivity of different facets of the cerium oxide crystals may be exploited (see the shape-selective synthetic methods[101–103]). New kinds of enzyme-like catalysts, namely single-atom nanozymes, may provide better accessibility of the active site to the substrate molecules. Some of them exhibit excellent catalytic performance surpassing that of natural enzymes,[104–106] but their ability to catalyse the phosphoester cleavage reactions has been only rarely exploited. A significant improvement in the catalytic efficiency may be achieved by decorating the inorganic "core" with suitable ligands or other structures emulating the functionalities of enzymes. The strategy for the design and synthesis of this kind of artificial enzyme was described in detail by Ginovska *et al.*,[107] who emphasized that not only the catalytic active site, but the whole enzyme scaffold should be emulated. A properly designed enzyme should not only support the (static) structural similarity with the established model substance, but also facilitate the tuning of the reaction conditions along the reaction trajectory.[108] However, there is a risk that this kind of bio-

decorated nanozyme will lose some of the advantages of nanozymes, *e.g.* resistance against extreme reaction conditions. The valuable discussion to this point can be found in the "viewpoint" article of Lyu and Scrimin.[62]

The key part of the catalytic cycle is the conversion of substrate (reactant) to product (part b) in Fig. 1. It is advantageous if the metal cations are involved in dephosphorylation reactions. Many enzymes (metalloenzymes) contain in their structure one or more metal cations. There is diversity in the possible metal ions present and their contribution to the enzyme's function.[109,110] Some metalloenzymes employ metal ions mainly for the purpose of substrate binding/stabilization, for instance enzymes working with substrates containing phosphate groups utilize divalent cations, such as Mg^{2+} or Mn^{2+}, to compensate for the negative charge on the phosphate group.[111,112] More often, however, metalloenzymes employ the unique properties of metal ions directly in the reaction. In some cases, replacement of the metal ion may be tolerated at the cost of reduced efficiency, for example replacing Mn^{2+} with Mg^{2+},[113] but typically metalloenzymes are uniquely specific in the metal ion they use. This is achieved by the specific arrangement of amino acids in the active site that precisely correspond to the coordination sphere requirements of the given metal ion.[114]

The different strategy is used in dephosphorylating reactions catalysed by cerium oxide, when the redox-cycling ability of cerium cations is combined with the unique properties of the cerium oxide crystalline lattice (see Fig. 5). Dynamic active sites[115,116] on the surface of cerium oxide consist of Ce^{4+}, Ce^{3+} and the –OH group (or the tightly attached water molecule). The coordination with the Ce^{4+} cation reduces the electron density on the P atom in the phosphate group, making it more susceptible to the nucleophilic attack with the –OH group. The –OH groups bound to the trivalent cation are stronger nucleophiles than the same groups bound to the tetravalent cation; therefore the presence of a certain amount of Ce^{3+} cations in the surface layer is essential for the proper function of ceria-based nanozymes. However, the Ce^{3+} concentration (most probably) is not the rate limiting parameter. The easy creation of oxygen vacancies[117–119] and their mobility in the cerium oxide structure[120–122] allow the re-creation of active sites virtually at any place on the surface of cerium oxide, where the hydroxyl group or an activated water molecule is present. The diffusion of oxygen through cerium oxide occurs *via* the vacancy hopping mechanism.[123]

The proposed mechanism is not fully validated yet, but it explains some discrepancies appearing in the literature, regarding *e.g.* the role of cerium valence in the catalytic process. There are also some experimental findings supporting its validity:

– The dephosphorylating activity of cerium oxide depends on the temperature during its preparation; for cerium oxide prepared by annealing at temperatures above *ca.* 600 °C the dephosphorylating activity decreases steeply with increasing temperature, and correlates strongly with the content of the –OH groups on its surface,[67] obviously in agreement with the proposed mechanism.

– When the precipitation/calcination method is used for cerium oxide preparation, its activity depends only slightly on the annealing temperature in a relatively broad range (*ca.* 300–600 °C), regardless of the precursor used. When the annealing temperature decreases below a certain limit, cerium oxide obtained by annealing the carbonate or oxalate precursors loses its activity completely, whereas cerium oxide prepared from cerium hydroxide retains a (certain) activity over the whole temperature range. From these observations, the necessity of the presence of a crystalline form of cerium oxide was deduced. When carbonate or oxalate is used as a precursor, its crystalline structure must be destroyed first, and subsequently cerium oxide is created. When the wet method (precipitation of cerium hydroxide by ammonia) is used, the mechanism of the cerium oxide creation is quite different; crystalline cerium oxide may be created at virtually any temperature.

– The proposed reaction mechanism is consistent with the results of the repeated use of cerium oxide in various solvents.[74] Briefly in aqueous solutions, cerium oxide can be used repeatedly, because the surface –OH groups on its surface can be re-generated by interactions with the molecules of water. In organic solvents (non-polar, aprotic), the –OH groups are consumed during the dephosphorylation

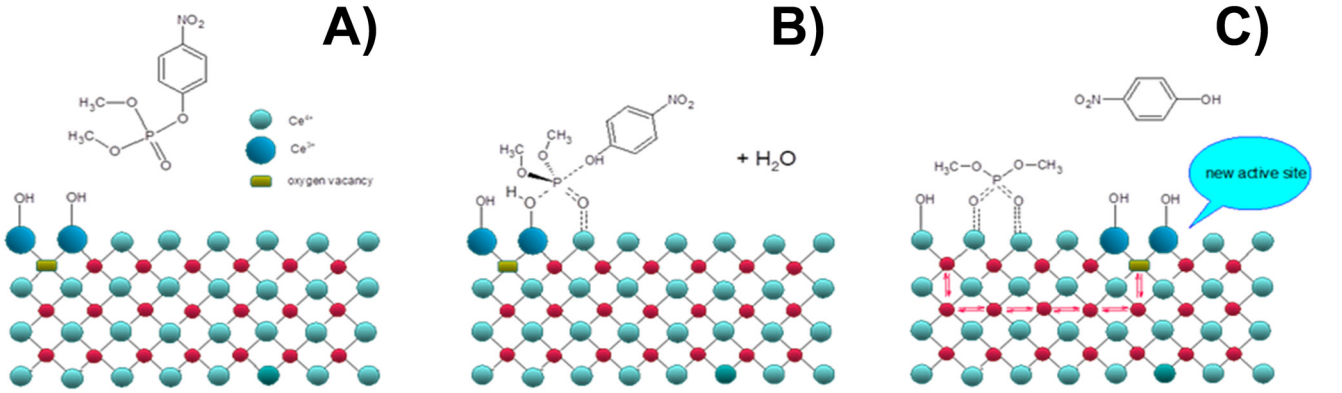


Fig. 5 The mechanism of the active site re-creation *via* oxygen vacancy migration (from ref. 74).

reaction. The ability of cerium oxide to destroy (toxic) organophosphates in nonpolar solvents is excellent, but it reduces rapidly in repeated cycles;[74] cerium oxide should be called a "reactive sorbent" instead of a catalyst in these applications (see above).

– The dephosphorylating activity of cerium oxide is demonstrated mainly in its nanocrystalline forms, but it is not restricted to them. Indeed, we found some (non-negligible) activity also in the case of certain technical materials, not declared as "nano".[74]

– None of the other examined metal oxides exhibited dephosphorylating activity towards cAMP comparable to cerium oxide.[74]

In connection with the described mechanism of action of cerium oxide, several questions may arise: (i) is cerium oxide, which is used in large quantities in some industries, capable of posing any risks to human health? The answer is not clear-cut. Indeed, some dephosphorylation activity was observed even with cerium oxides that were not declared as "nanoceria," including technical materials such as glass polishing powders.[74] Independently, Xu *et al.*[124] confirmed the ability of cerium oxide to cleave DNA molecules. These facts deserve attention. On the other hand, cerium oxide for industrial applications, such as chemical mechanical glass polishing, is produced at temperatures around 1000 °C,[125] under conditions where it loses its dephosphorylation activity. (ii) Can the described mechanism influence the fate of chemical pollutants (*e.g.*, pesticides) in the environment? Hydrolysis and microbial decomposition are the primary mechanisms for the degradation of organic pollutants in soils and aquatic environments, with metal ions and metal oxides playing crucial roles.[126] However, cerium oxide does not occur in pure form in significant quantities in the environment and likely does not exert a significant influence on these processes. In contrast, iron oxides are plentiful in the environment. Iron cations possess redox-switching abilities. Nonetheless, iron oxides exist in numerous crystalline forms, making it considerably more challenging to delineate the precise degradation mechanism compared to cerium oxide. The same holds true for other oxides containing a metal cation with redox switching ability (*e.g.* manganese). The described mechanism can occur in nature, but it is difficult to observe.

Oxygen vacancies and their role in the cerium oxide activity

Oxygen vacancies are non-stoichiometric point defects frequently occurring on the surface of metal oxides[127,128] that significantly affect their properties and catalytic performance.[129] Oxygen vacancies govern the catalytic properties of metal oxide-based catalysts including their surface chemistry (presence of OH groups, wettability), electron transfer or photocatalytic efficiency.[130] The presence of oxygen vacancies in cerium oxide is favoured by the redox switching ability of cerium cations,[131,132] while the formation energy of oxygen vacancies is strongly influenced by the nanocrystalline structure of cerium oxide.[119] As follows from theoretical considerations,[133] the simple (fluorite-like) structure of cerium oxide not only enables the creation of oxygen vacancies, but also supports their surface-bulk migration,[134] which is responsible for the antioxidant activity of cerium oxide (and probably also for some unexpected magnetic properties of cerium oxide[135]). X-ray photoelectron spectroscopy (XPS) is one of the most frequently used methods for element speciation. It reflects the composition of the surface layer of cerium oxide, which is relevant for the assessment of the (nano)ceria catalytic activity.[136] In principle, the oxidation state of cerium is governed by the partial oxygen pressure in the surrounding environment,[137,138] but it may be affected by varying the synthetic conditions, or by doping with polyvalent metal cations.[139] There are still extensive discussions in the literature regarding the effect of the Ce^{3+}/Ce^{4+} ratio on the catalytic efficiency of cerium oxide (and also the reliability of the Ce^{3+} determination by XPS and other methods[140–142]). We postulated that both trivalent and tetravalent cerium cations contribute to the dephosphorylating activity of cerium oxide, but their exact ratio is not the limiting parameter, as it may be adjusted (in the respective active site) by oxygen vacancy migration. Vacancy migration is an integral part of the phosphatase-mimetic catalytic cycle.[74] This implies that not only the redox switching ability of the metal (cerium) cation, but also the simple and robust crystalline structure of cerium oxide is beneficial. In nanozymatic applications, the requirements for metal oxide properties present a challenge marked by apparent contradictions. While crystalline defects are favorable for the formation of active centers, maintaining the structural integrity of the oxide is crucial for facilitating the migration of these active centers. Cerium oxide emerges as a promising candidate, as its robust fluorite-like crystalline structure enables oxygen transport through the electron (polaron) hopping mechanism.[143]

Conclusions and expected trends

In this article, we presented a dual view on the same kind of reaction – a hydrolytic cleavage of the phosphoester bond in organophosphate compounds – with the goal of finding analogies that could be exploited in the design of (bio) catalysts suitable for industrial applications of these reactions. We compared metal oxide-based (mainly ceria-based) nanozymes with their protein-based counterparts – metalloenzymes.

Materials with phosphatase-mimetic ability are desirable not only for numerous applications in bioscience and medicine, but also they can become the basis of new industries if they are available in sufficient quantities, with consistent quality and at a reasonable price. A recovery of phosphorus from phosphorylated biomolecules (phospholipids, DNA) suggested by Manto *et al.*[144] may serve as an example. For those applications, catalysts resistant against extreme conditions are especially needed.

Natural enzymes have been evolutionarily optimized for the conditions (temperature, pressure, pH) present inside living cells. At elevated temperature, most of the natural enzymes start to unfold and denature. There is an optimum temperature for a given enzyme activity.[145] The temperature (solvent, pH) tolerance of an enzyme can be improved by means of protein engineering, in which mutations are introduced into the protein scaffold of the enzyme that stabilize the protein without affecting its enzymatic activity.[146,147] Immobilization on inorganic (nano)particles with some additional functionality (*e.g.* the separability in a magnetic field) not only enhances the applicability of

enzymes,[148] but may also improve their thermal resistance.[149] Despite that, the accessible range of conditions is rather restricted, compared to nanozymes, and the range of possible industrial applications remains seriously limited.

At present, mainly Ce- and Zr-based nanozymes are used as dephosphorylating agents.[15] The mechanism of organophosphate hydrolysis catalysed by cerium oxide was proposed, based on the concept of dynamic active sites. This concept is transferable to other metal oxides containing cations with redox switching ability – Fe, Mn, Cu, Co. Oxides of these metals occur in various forms (crystalline phases); therefore the effects caused by this mechanism are hard to observe. On the other hand, once identified (formulated), it can be more easily detected in nature (with potential implications in the theory of pollutant transformation or even in the theory of life origin on Earth) and exploited in industry.

For industrial applications, there is an urgent need for more effective nanocatalysts (nanozymes). Various synthesis methods are available to prepare cerium oxide with desired properties, and numerous characterization methods exist for its detailed characterization. However, a clear relationship between certain measurable characteristics of cerium oxide (descriptors) and its performance characteristics (*e.g.*, phosphatase-like ability) is currently lacking. A systematic approach utilizing methods of defect[120–122]/vacancy[134,150,151] engineering, supported by effective computational methods (which are currently lacking for these complex systems), could facilitate the development of scalable industrially applicable procedures (probably hydrothermal). However, it is unlikely that the efficiency of such nanozymes will surpass that of natural enzymes.

For specific purposes, cerium oxide can be applied in the form of a thin layer on a suitable carrier, such as low-cost sorbents, magnetic cores[152] or nylon nanofibres.[153] This trend, combined with the systematic exploitation of the multifunctionality of certain nanozymes (with cerium oxide at the forefront), may improve the applicability of metal oxide-based catalysts, although not necessarily their efficiency. In general, composites consisting of several components with different functionalities are very promising materials with diverse potential applications – see the combination of cerium oxide (capable of detoxifying organophosphate compounds) with titanium oxide, which eliminates residual organic pollutants exploiting its photocatalytic ability.[154]

Protein engineering

Naturally occurring enzymes face challenges when applied in abiological settings in terms of their stability, their narrow range of substrates or difficulty of their production. For some reactions of interest there may not even be naturally occurring enzymes available. Protein engineering is a field that aims to address these issues in order to modify existing enzymes and optimize their properties; or generate new enzymes with novel activities/functions.[155,156]

When optimizing an enzyme for particular applications, one would start from an existing enzyme that shows at least some activity towards the chemical reaction of interest. Such an enzyme would then undergo several rounds of iterative optimization procedure where random mutations in the amino acid sequence are introduced, and the properties of the mutated enzymes are verified before accepting or rejecting the changed enzyme sequences for the next round. This approach is called directed evolution (DE) since it mimics the natural selection process.[157,158] The selection criterion can be either the catalytic activity or protein stability, when sufficient activity has been already reached. Limitations of DE include the fact that highly specialized assay is required for the high-throughput screening of mutated enzyme variants and that DE will typically result in optimized enzymes that are not that distant to the original in terms of their sequence, structure and properties.[158]

Alternatives to DE are rational design approaches that introduce changes in the enzyme purposefully based on insight into its function, instead of relying on random mutations. With sufficient understanding of the structure and interaction of the substrate binding to the enzyme, it may be possible to suggest targeted mutations to the active site to accommodate a different substrate.[156,159] Computational approaches can help suggest optimal mutations in the protein scaffold, far away from the active site, in order to increase enzyme stability.[160,161] Rational approaches and DE can be combined in a 'semi-rational' fashion, in which the mutations are not fully random, but targeted onto a focused region of the enzyme.[162–164]

Chimeragenesis can be used when bigger 'jumps' in the protein structure are required. By combining structural elements from different proteins, it generates new 'chimeric' protein structures. Some level of insight and or analysis is required to understand which protein elements/substructures are suitable for this 'shuffling'.[165,166] In the need of completely novel enzymatic function, one can exploit the immune system to generate antibodies against a surrogate molecule representing the transition state of the chemical reaction of interest. The resulting antibody, exhibiting low level catalytic activity, can then be further optimized by the aforementioned methods.[167–169]

The recent advent of AI-based protein structure prediction methods, spearheaded by AlphaFold[170] and RoseTTAfold,[171] opened the doors to more avenues in protein engineering. Based on just its sequence it is now possible to predict, with reasonable accuracy, the structure of an enzyme in the absence of an experimental structure. This enables rational design approaches where they were not possible before. Beyond that, AI-based methods can be used to derive completely new protein structures, different from naturally occurring scaffolds[37–39] with potential for novel enzymatic functions, though they would still need to undergo more traditional optimization steps described above.

A substantial enhancement of the nanozyme selectivity may be achieved by molecular imprinting methods,[172–174] whereas covering nanoparticles with hydrogels[175–177] facilitate their applications in biomedia. On the way between simple inorganic systems with the ability to mimic natural enzymes and real protein-based enzymes, there are a number of organometallic[178] and other systems (vesicular,[179] supramolecular[180–183]). They often contain the same or similar structural units as the natural enzymes. It is also possible to embed small molecule catalysts into otherwise inert protein scaffolds (like artificial cofactors).[184,185] With detailed knowledge of enzymatic reaction mechanisms and

using protein engineering methods, the design of materials with enzyme-mimetic activity and enhanced durability, less susceptible to the conditions outside the living cell, could be possible.

Although this article has been devoted almost exclusively to nanozymatically accelerated dephosphorylation reactions, the most recent studies demonstrated that cerium oxide is also effective in the hydrolytic cleavage of other emerging pollutants, *e.g.* sulfonamides.[186]

It's quite clear by now that machine learning or AI approaches will play an increasingly more important role in the field of (bio)catalyst design. The ability of AI-based protein structure prediction methods (AlphaFold,[170] RoseTTAFold,[171] and others[187]) to generate reliable structures from just the amino acid sequence has led to a revolution in protein engineering of enzymes.[160,188] There is now a plethora of machine learning methods that can aid in the various steps in enzyme optimization.[189,190] Potentially even more exciting is the possibility to generate completely new protein structures divergent from the naturally occurring ones,[37–39] thus opening doors to the potential of artificial enzymes with novel activities. Applications of AI in the fields of materials science and heterogeneous catalysis are no less exciting.[40] Recent studies, using AI approaches, were able to design thousands of potential novel materials[191] as well as autonomously propose workflows to synthetize them.[192] Machine learning approaches can also speed up traditionally demanding calculations of catalytic mechanisms and/or catalyst properties allowing computations on bigger more realistic systems giving us better understanding and directions for future designs.[193–195] The question that remains to be answered is whether and how AI approaches can 'bridge' the worlds of enzymatic biocatalysis and inorganic nanozymes to derive new generation of bio-inspired nanocatalysts.

Author contributions

Both authors contributed equally to this article.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The assistance provided by the NanoEnviCz Research Infrastructure supported by the Ministry of Education, Youth and Sports of the Czech Republic, project No. LM2015073, is acknowledged. The financial support for the project No. CZ.02.1.01/0.0/0.0/17_048/0007399 from the same provider is acknowledged. PJ Jr. thanks the PNRR: National Center for Gene Therapy and Drugs based on RNA Technology CUP B83C22002860006 CN_0000004 for financial support. Dr. Martin Šťastný is thanked for technical assistance and valuable discussions during the manuscript preparation.

References

1 L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, Intrinsic peroxidase-like activity of ferromagnetic nanoparticles, *Nat. Nanotechnol.*, 2007, **2**, 577–583.
2 J. Sumaoka, M. Yashiro and M. Komiyama, Remarkably fast hydrolysis of 3′,5′-cyclic adenosine monophosphate by cerium(III) hydroxide cluster, *J. Chem. Soc., Chem. Commun.*, 1992, 1707–1708.
3 S. Scott, H. Zhao, A. Dey and T. B. Gunnoe, Nano-Apples and Orange-Zymes, *ACS Catal.*, 2020, **10**, 14315–14317.
4 H. Wei and E. Wang, Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes, *Chem. Soc. Rev.*, 2013, **42**, 6060–6093.
5 Y. Lin, J. Ren and X. Qu, Catalytically Active Nanomaterials: A Promising Candidate for Artificial Enzymes, *Acc. Chem. Res.*, 2014, **47**, 1097–1105.
6 Y. Huang, J. Ren and X. Qu, Nanozymes: Classification, Catalytic Mechanisms, Activity Regulation, and Applications, *Chem. Rev.*, 2019, **119**, 4357–4412.
7 X. Wang, W. Guo, Y. Hu, J. Wu and H. Wei, *Nanozymes: Next Wave of Artificial Enzymes*, Springer, Berlin, Heidelberg, 2016.
8 S. Ahmadi, K. Rahimizadeh, A. Shafiee, N. Rabiee and S. Iravani, Nanozymes and their emerging applications in biomedicine, *Process Biochem.*, 2023, **131**, 154–174.
9 Z. Chen, Y. Yu, Y. Gao and Z. Zhu, Rational Design Strategies for Nanozymes, *ACS Nano*, 2023, **17**, 13062–13080.
10 R. Zhang, K. Fan and X. Yan, Nanozymes: created by learning from nature, *Sci. China: Life Sci.*, 2020, **63**, 1183–1200.
11 S. Singh, Cerium oxide based nanozymes: Redox phenomenon at biointerfaces, *Biointerphases*, 2016, **11**, 04B202.
12 K. Reed, A. Cormack, A. Kulkarni, M. Mayton, D. Sayle, F. Klaessig and B. Stadler, Exploring the properties and applications of nanoceria: is there still plenty of room at the bottom?, *Environ. Sci.: Nano*, 2014, **1**, 390–405.
13 A. S. Karakoti, N. A. Monteiro-Riviere, R. Aggarwal, J. P. Davis, R. J. Narayan, W. T. Seif, J. McGinnis and S. Seal, Nanoceria as antioxidant: Synthesis and biomedical applications, *JOM*, 2008, **60**, 33–37.
14 Y. Ma, Z. Tian, W. Zhai and Y. Qu, Insights on catalytic mechanism of CeO_2 as multiple nanozymes, *Nano Res.*, 2022, **15**, 10328–10342.
15 Y. Wu, W. Chen, C. Wang and D. Xing, Overview of nanozymes with phosphatese-like activity, *Biosens. Bioelectron.*, 2023, **237**, 115470.
16 M. Komiyama, T. Kodama, N. Takeda, J. Sumaoka, T. Shiiba, Y. Matsumoto and M. Yashiro, Catalytically active species for CeCl3-induced DNA hydrolysis, *J. Biochem.*, 1994, **115**, 809–810.
17 M. Komiyama and J. Sumaoka, Progress towards synthetic enzymes for phosphoester hydrolysis, *Curr. Opin. Chem. Biol.*, 1998, **2**, 751–757.

18 M. Komiyama, Ce-based solid-phase catalysts for phosphate hydrolysis as new tools for next-generation nanoarchitectonics, *Sci. Technol. Adv. Mater.*, 2023, **24**(1), 2250705.

19 B. Zhu, Y.-J. Wu, D.-Q. Zhao and J.-Z. Ni, Cleavage of nucleotides by Ce^{4+} and the lanthanide metal complexes, *BioMetals*, 1999, **12**, 11–17.

20 K. G. Ragunathan and H.-J. Schneider, Binuclear Lanthanide Complexes as Catalysts for the Hydrolysis of Bis(p-nitrophenyl)-phosphate and Double-Stranded DNA, *Angew. Chem., Int. Ed. Engl.*, 1996, **35**, 1219–1221.

21 S. T. Lin and K. J. Klabunde, Thermally activated magnesium oxide surface chemistry. Adsorption and decomposition of phosphorus compounds, *Langmuir*, 1985, **1**, 600–605.

22 O. Koper and K. J. Klabunde, Destructive Adsorption of Chlorinated Hydrocarbons on Ultrafine (Nanoscale) Particles of Calcium Oxide. 3. Chloroform, Trichloroethene, and Tetrachloroethene, *Chem. Mater.*, 1993, **5**, 500–505, DOI: **10.1021/CM9703583**.

23 A. Khaleel, W. Li and K. J. Klabunde, Nanocrystals as stoichiometric reagents with unique surface chemistry. New adsorbents for air purification, *Nanostruct. Mater.*, 1999, **12**, 463–466.

24 A. Khaleel, P. N. Kapoor and K. J. Klabunde, Nanocrystalline metal oxides as new adsorbents for air purification, *Nanostruct. Mater.*, 1999, **11**, 459–468.

25 E. Lucas, S. Decker, A. Khaleel, A. Seitz, S. Fultz, A. Ponce, W. Li, C. Carnes and K. J. Klabunde, Nanocrystalline metal oxides as unique chemical reagents/sorbents, *Chemistry*, 2001, **7**, 2505–2510.

26 N. E. Zander, E. Kowalski, A. M. Rawlett and J. A. Orlicki, *Decontamination of chemical agent simulant by nanometal oxides*, Abrdeen, 2007.

27 V. Štengl, D. Králová, F. Opluštil and T. Němec, Mesoporous manganese oxide for warfare agents degradation, *Microporous Mesoporous Mater.*, 2012, **156**, 224–232.

28 V. Štengla, T. M. Grygar, F. Opluštil and T. Němec, Sulphur mustard degradation on zirconium doped Ti-Fe oxides, *J. Hazard. Mater.*, 2011, **192**, 1491–1504.

29 V. Štengl, V. Houšková, S. Bakardjieva, N. Murafa, M. Maříková, F. Opluštil and T. Němec, Zirconium doped nano-dispersed oxides of Fe, Al and Zn for destruction of warfare agents, *Mater. Charact.*, 2010, **61**, 1080–1088.

30 J. Henych, P. Janoš, M. Kormunda, J. Tolasz and V. Štengl, Reactive adsorption of toxic organophosphates parathion methyl and DMMP on nanostructured Ti/Ce oxides and their composites, *Arabian J. Chem.*, 2019, **12**, 4258–4269, DOI: **10.1016/j.arabjc.2016.06.002**.

31 P. Janoš, P. Kuráň, M. Kormunda, V. Štengl, T. M. Grygar, M. Došek, M. Štastný, J. Ederer, V. Pilařová and L. Vrtoch, Cerium dioxide as a new reactive sorbent for fast degradation of parathion methyl and some other organophosphates, *J. Rare Earths*, 2014, **32**, 360–370.

32 P. Janoš, P. Kuráň, J. Ederer, M. AŠastný, L. Vrtoch, M. Pšenička, J. Henych, K. Mazanec and M. Skoumal, Recovery of Cerium Dioxide from Spent Glass-Polishing Slurry and Its Utilization as a Reactive Sorbent for Fast Degradation of Toxic Organophosphates, *Adv. Mater. Sci. Eng.*, 2015, 211421, DOI: **10.1155/2015/241421**.

33 P. Janoš, J. Henych, O. Pelant, V. Pilařová, L. Vrtoch, M. Kormunda, K. Mazanec and V. Štengl, Cerium oxide for the destruction of chemical warfare agents: A comparison of synthetic routes, *J. Hazard. Mater.*, 2016, **304**, 259–268.

34 M. H. Kuchma, C. B. Komanski, J. Colon, A. Teblum, A. E. Masunov, B. Alvarado, S. Babu, S. Seal, J. Summy and C. H. Baker, Phosphate ester hydrolysis of biologically relevant molecules by cerium oxide nanoparticles, *Nanomedicine*, 2010, **6**, 738–744.

35 P. Janoš, J. Ederer and M. Došek, Some environmentally relevant reactions of cerium oxide, *Nova Biotechnol. Chim.*, 2014, **13**, 148–161, DOI: **10.1515/nbec-2015-0005**.

36 K. Binnemans and P. T. Jones, Rare Earths and the Balance Problem, *J. Sustain. Met.*, 2015, **1**, 29–38.

37 J. Dauparas, I. Anishchenko, N. Bennett, H. Bai, R. J. Ragotte, L. F. Milles, B. I. M. Wicky, A. Courbet, R. J. de Haas, N. Bethel, P. J. Y. Leung, T. F. Huddy, S. Pellock, D. Tischer, F. Chan, B. Koepnick, H. Nguyen, A. Kang, B. Sankaran, A. K. Bera, N. P. King and D. Baker, Robust deep learning–based protein sequence design using ProteinMPNN, *Science*, 2022, **378**, 49–56.

38 J. L. Watson, D. Juergens, N. R. Bennett, B. L. Trippe, J. Yim, H. E. Eisenach, W. Ahern, A. J. Borst, R. J. Ragotte, L. F. Milles, B. I. M. Wicky, N. Hanikel, S. J. Pellock, A. Courbet, W. Sheffler, J. Wang, P. Venkatesh, I. Sappington, S. V. Torres, A. Lauko, V. De Bortoli, E. Mathieu, S. Ovchinnikov, R. Barzilay, T. S. Jaakkola, F. DiMaio, M. Baek and D. Baker, De novo design of protein structure and function with RFdiffusion, *Nature*, 2023, **620**, 1089–1100.

39 S. Alamdari, N. Thakkar, R. van den Berg, A. X. Lu, N. Fusi, A. P. Amini and K. K. Yang, Protein generation with evolutionary diffusion: sequence is all you need, *bioRxiv*, 2023, preprint, DOI: **10.1101/2023.09.11.556673**.

40 H. Li, Y. Jiao, K. Davey and S. Z. Qiao, Data-Driven Machine Learning for Understanding Surface Structures of Heterogeneous Catalysts, *Angew. Chem., Int. Ed.*, 2023, **62**, e202216383, DOI: **10.1002/anie.202216383**.

41 A. Dhall and W. Self, Cerium Oxide Nanoparticles: A Brief Review of Their Synthesis Methods and Biomedical Applications, *Antioxidants*, 2018, **7**, 97.

42 S. Kontham, K. Mandava, S. Dosa, F. U. Mohd, O. A. Mohammed and A. U. Mohammad, Review on facile synthesis of cerium oxide nanoparticles and their biomedical applications, *Inorg. Nano-Met. Chem.*, 2022, **52**, 1183–1195.

43 H. Nosrati, M. Heydari and M. Khodaei, Cerium oxide nanoparticles: Synthesis methods and applications in wound healing, *Mater. Today Bio*, 2023, **23**, 100823.

44 P. Janoš and M. Petrák, Preparation of ceria-based polishing powders from carbonates, *J. Mater. Sci.*, 1991, **26**, 4062–4066.

45 P. Janoš, J. Ederer, V. Pilařová, J. Henych, J. Tolasz, D. Milde and T. Opletal, Chemical mechanical glass polishing

with cerium oxide: Effect of selected physico-chemical characteristics on polishing efficiency, *Wear*, 2016, **362–363**, 114–120, DOI: **10.1016/j.wear.2016.05.020**.

46 L. M. Dennis and W. H. Magee, Contributions to the chemistry of cerium, *J. Am. Chem. Soc.*, 1894, **16**, 649–664.

47 J. Henych, V. Štengl, M. Slušná, T. Matys Grygar, P. Janoš, P. Kuráň and M. Šťastný, Degradation of organophosphorus pesticide parathion methyl on nanostructured titania-iron mixed oxides, *Appl. Surf. Sci.*, 2015, **344**, 9–16, DOI: **10.1016/j.apsusc.2015.02.181**.

48 J. Henych, P. Janoš, M. Kormunda, J. Tolasz and V. Štengl, Reactive adsorption of toxic organophosphates Parathion methyl and DMMP on nanostructured Ti/Ce oxides and their composites, *Arabian J. Chem.*, 2019, **12**, 4258–4269, DOI: **10.1016/j.arabjc.2016.06.002**.

49 K. Nakagawa, Y. Tezuka, T. Ohshima, M. Katayama, T. Ogata, K.-I. Sotowa, M. Katoh and S. Sugiyama, Formation of cerium carbonate hydroxide and cerium oxide nanostructures by self-assembly of nanoparticles using surfactant template and their catalytic oxidation, *Adv. Powder Technol.*, 2016, **27**, 2128–2135.

50 S. Barkam, J. Ortiz, S. Saraf, N. Eliason, R. Mccormack, S. Das, A. Gupta, C. Neal, A. Petrovici, C. Hanson, M. D. Sevilla, A. Adhikary and S. Seal, Modulating the Catalytic Activity of Cerium Oxide Nanoparticles with the Anion of the Precursor Salt, *J. Phys. Chem. C*, 2017, **121**, 20039–20050.

51 S. Sehar, I. Naz, A. Rehman, W. Sun, S. S. Alhewairini, M. N. Zahid and A. Younis, Shape-controlled synthesis of cerium oxide nanoparticles for efficient dye photodegradation and antibacterial activities, *Appl. Organomet. Chem.*, 2021, **35**, e6069.

52 Q. Yuan, H. H. Duan, L. Le Li, L. D. Sun, Y. W. Zhang and C. H. Yan, Controlled synthesis and assembly of ceria-based nanomaterials, *J. Colloid Interface Sci.*, 2009, **335**, 151–167.

53 M. Komiyama, Ce-based solid-phase catalysts for phosphate hydrolysis as new tools for next-generation nanoarchitectonics, *Sci. Technol. Adv. Mater.*, 2023, **24**, 2250705.

54 G. Schenk, N. Mitić, G. R. Hanson and P. Comba, Purple acid phosphatase: A journey into the function and mechanism of a colorful enzyme, *Coord. Chem. Rev.*, 2013, **257**, 473–482.

55 C. B. Castro, M. P. Ferreira and C. G. Marques Netto, Metalloenzyme mechanisms correlated to their turnover number and metal lability, *Curr. Res. Chem. Biol.*, 2021, **1**, 100004, DOI: **10.1016/j.crchbi.2021.100004**.

56 C. Selleck, D. Clayton, L. R. Gahan, N. Mitić, R. P. McGeary, M. M. Pedroso, L. W. Guddat and G. Schenk, Visualization of the Reaction Trajectory and Transition State in a Hydrolytic Reaction Catalyzed by a Metalloenzyme, *Chem. – Eur. J.*, 2017, **23**, 4778–4781.

57 D. Feder, L. R. Gahan, R. P. McGeary, L. W. Guddat and G. Schenk, The Binding Mode of an ADP Analogue to a Metallohydrolase Mimics the Likely Transition State, *ChemBioChem*, 2019, **20**, 1536–1540.

58 R. Ragg, M. N. Tahir and W. Tremel, Solids Go Bio: Inorganic Nanoparticles as Enzyme Mimics, *Eur. J. Inorg. Chem.*, 2016, **2016**, 1906–1915.

59 X.-L. Huang, Hydrolysis of Phosphate Esters Catalyzed by Inorganic Iron Oxide Nanoparticles Acting as Biocatalysts, *Astrobiology*, 2018, **18**, 294–310.

60 X.-L. Huang, Iron Oxide Nanoparticles: An Inorganic Phosphatase, *Nanocatalysts*, InterOpen, 2019, DOI: **10.5772/INTECHOPEN.82650**.

61 D. Kühnel, C. Marquardt, K. Nau, H. F. Krug, B. Mathes and C. Steinbach, Environmental impacts of nanomaterials: providing comprehensive information on exposure, transport and ecotoxicity - the project DaNa2.0, *Environ. Sci. Eur.*, 2014, **26**, 21.

62 Y. Lyu and P. Scrimin, Mimicking Enzymes: The Quest for Powerful Catalysts from Simple Molecules to Nanozymes, *ACS Catal.*, 2021, **11**, 11501–11509.

63 A. J. Kirby and F. Hollfelder, *From Enzyme Models to Model Enzymes*, RSC Publishing, 2009.

64 S. Kozuch and J. M. L. Martin, 'Turning over' definitions in catalytic cycles, *ACS Catal.*, 2012, **2**, 2787–2794.

65 M. M. Pedroso, F. Ely, N. Mitić, M. C. Carpenter, L. R. Gahan, D. E. Wilcox, J. L. Larrabee, D. L. Ollis and G. Schenk, Comparative investigation of the reaction mechanisms of the organophosphate-degrading phosphotriesterases from Agrobacterium radiobacter (OpdA) and Pseudomonas diminuta (OPH), *J. Biol. Inorg. Chem.*, 2014, **19**, 1263–1275.

66 S. D. Aubert, Y. Li and F. M. Raushel, Mechanism for the Hydrolysis of Organophosphates by the Bacterial Phosphotriesterase, *Biochemistry*, 2004, **43**, 5707–5715.

67 J. Ederer, P. Janoš, M. Šťastný, J. Henych, K. Ederer, M. Š. Slušná and J. Tolasz, Nanocrystalline cerium oxide for catalytic degradation of paraoxon methyl: Influence of CeO_2 surface properties, *J. Environ. Chem. Eng.*, 2021, **9**, 106229.

68 K. S. Hadler, N. Mitić, F. Ely, G. R. Hanson, L. R. Gahan, J. A. Larrabee, D. L. Ollis and G. Schenk, Structural flexibility enhances the reactivity of the bioremediator glycerophosphodiesterase by fine-tuning its mechanism of hydrolysis, *J. Am. Chem. Soc.*, 2009, **131**, 11900–11908.

69 O. V. Safonova, A. A. Guda, C. Paun, N. Smolentsev, P. M. Abdala, G. Smolentsev, M. Nachtegaal, J. Szlachetko, M. A. Soldatov, A. V. Soldatov and J. A. van Bokhoven, Electronic and Geometric Structure of Ce 3+ Forming Under Reducing Conditions in Shaped Ceria Nanoparticles Promoted by Platinum, *J. Phys. Chem. C*, 2014, **118**, 1974–1982.

70 Y. Fu, Y. Zhang, F. Fan, B. Wang and Z. Cao, Degradation of pesticides diazinon and diazoxon by phosphotriesterase: insight into divergent mechanisms from QM/MM and MD simulations, *Phys. Chem. Chem. Phys.*, 2022, **24**, 687–696.

71 A. A. Vernekar, T. Das and G. Mugesh, Vacancy-Engineered Nanoceria: Enzyme Mimetic Hotspots for the Degradation of Nerve Agents, *Angew. Chem., Int. Ed.*, 2016, **55**, 1412–1416.

72 K. Khulbe and G. Mugesh, Lock and key-based nanozyme model to understand the substituent effect on the hydrolysis of organophosphate-based nerve agents by Zr-incorporated cerium oxide, *Polyhedron*, 2019, **172**, 198–204.

73 J. Chin and X. Zou, Catalytic hydrolysis of cAMP, *Can. J. Chem.*, 1987, **65**, 1882–1884.

74 P. Janoš, J. Ederer, M. Došek, J. Štojdl, J. Henych, J. Tolasz, M. Kormunda and K. Mazanec, Can cerium oxide serve as a phosphodiesterase-mimetic nanozyme?, *Environ. Sci.: Nano*, 2019, **6**, 3684–3698.

75 M. Wall, B. Linkletter, D. Williams, A. M. Lebuis, R. C. Hynes and J. Chin, Rapid hydrolysis of 2′,3′-cAMP with a Cu(II) complex: Effect of intramolecular hydrogen bonding on the basicity and reactivity of a metal- bound hydroxide, *J. Am. Chem. Soc.*, 1999, **121**, 4710–4711.

76 S. Dagar, S. Sarkar and S. Rajamani, Nonenzymatic Template-Directed Primer Extension Using 2′-3′ Cyclic Nucleotides Under Wet-Dry Cycles, *Origins Life Evol. Biospheres*, 2023, **53**, 43–60.

77 M. M. Pedroso, J. A. Larrabee, F. Ely, S. E. Gwee, N. Mitic, D. L. Ollis, L. R. Gahan and G. Schenk, CaII Binding Regulates and Dominates the Reactivity of a Transition-Metal-Ion-Dependent Diesterase from Mycobacterium tuberculosis, *Chem. – Eur. J.*, 2016, **22**, 999–1009.

78 K. Y. Wong and J. Gao, Insight into the phosphodiesterase mechanism from combined QM/MM free energy simulations, *FEBS J.*, 2011, **278**, 2579–2595.

79 R. X. Xu, W. J. Rocque, M. H. Lambert, D. E. Vanderwall, M. A. Luther and R. T. Nolte, Crystal structures of the catalytic domain of phosphodiesterase 4B complexed with amp, 8-Br-AMP, and rolipram, *J. Mol. Biol.*, 2004, **337**, 355–365.

80 A. M. Ashrafi, Z. Bytesnikova, J. Barek, L. Richtera and V. Adam, A critical comparison of natural enzymes and nanozymes in biosensing and bioassays, *Biosens. Bioelectron.*, 2021, **192**, 113494.

81 B. Jiang, D. Duan, L. Gao, M. Zhou, K. Fan, Y. Tang, J. Xi, Y. Bi, Z. Tong, G. F. Gao, N. Xie, A. Tang, G. Nie, M. Liang and X. Yan, Standardized assays for determining the catalytic activity and kinetics of peroxidase-like nanozymes, *Nat. Protoc.*, 2018, **13**, 1506–1520.

82 C. Vogt and B. M. Weckhuysen, The concept of active site in heterogeneous catalysis, *Nat. Rev. Chem.*, 2022, **6**, 89–111.

83 G. B. Smejkal and S. Kakumanu, Enzymes and their turnover numbers, *Expert Rev. Proteomics*, 2019, **16**, 543–544.

84 M. Zandieh and J. Liu, Surface Science of Nanozymes and Defining a Nanozyme Unit, *Langmuir*, 2022, **38**, 3617–3622.

85 M. Zandieh and J. Liu, Nanozymes: Definition, Activity, and Mechanisms, *Adv. Mater.*, 2023, 2211041, DOI: **10.1002/ADMA.202211041**.

86 A. C. R. Martin, C. A. Orengo, E. G. Hutchinson, S. Jones, M. Karmirantzou, R. A. Laskowski, J. B. O. Mitchell, C. Taroni and J. M. Thornton, Protein folds and functions, *Structure*, 1998, **6**, 875–884.

87 J. M. Thornton, C. A. Orengo, A. E. Todd and F. M. G. Pearl, Protein folds, functions and evolution, *J. Mol. Biol.*, 1999, **293**, 333–342.

88 R. Powers, J. C. Copeland, K. Germer, K. A. Mercier, V. Ramanatlian and P. Revesz, Comparison of protein active site structures for functional annotation of proteins and drug design, *Proteins: Struct., Funct., Bioinf.*, 2006, **65**, 124–135.

89 A. Warshel, P. K. Sharma, M. Kato, Y. Xiang, H. Liu and M. H. M. Olsson, Electrostatic Basis for Enzyme Catalysis, *Chem. Rev.*, 2006, **106**, 3210–3235.

90 K. E. Ranaghan, L. Ridder, B. Szefczyk, W. A. Sokalski, J. C. Hermann and A. J. Mulholland, Transition state stabilization and substrate strain in enzyme catalysis: ab initio QM/MM modelling of the chorismate mutase reaction, *Org. Biomol. Chem.*, 2004, **2**, 968–980.

91 N. Furnham, G. L. Holliday, T. A. P. De Beer, J. O. B. Jacobsen, W. R. Pearson and J. M. Thornton, The Catalytic Site Atlas 2.0: Cataloging catalytic sites and residues identified in enzymes, *Nucleic Acids Res.*, 2014, **42**, 485–489.

92 A. Warshel, Energetics of enzyme catalysis, *Proc. Natl. Acad. Sci. U. S. A.*, 1978, **75**, 5250–5254.

93 T. K. Harris and G. J. Turner, Structural Basis of Perturbed pKa Values of Catalytic Groups in Enzyme Active Sites, *IUBMB Life*, 2002, **53**, 85–98.

94 D. Blaha-Nelson, D. M. Krüger, K. Szeler, M. Ben-David and S. C. L. Kamerlin, Active Site Hydrophobicity and the Convergent Evolution of Paraoxonase Activity in Structurally Divergent Enzymes: The Case of Serum Paraoxonase 1, *J. Am. Chem. Soc.*, 2017, **139**, 1155–1167.

95 L. Agosta, D. Arismendi-Arrieta, M. Dzugutov and K. Hermansson, Origin of the Hydrophobic Behaviour of Hydrophilic CeO_2, *Angew. Chem., Int. Ed.*, 2023, **62**, e202303910.

96 D. Zhu, W. Liu, R. Zhao, Z. Shi, X. Tan, Z. Zhang, Y. Li, L. Ji and X. Zhang, Microscopic insights into hydrophobicity of cerium oxide: Effects of crystal orientation and lattice constant, *J. Mater. Sci. Technol.*, 2022, **109**, 20–29.

97 P. Janoš, J. Henych, J. Pfeifer, N. Zemanová, V. Pilařová, D. Milde, T. Opletal, J. Tolasz, M. Malý and V. Štengl, Nanocrystalline cerium oxide prepared from a carbonate precursor and its ability to breakdown biologically relevant organophosphates, *Environ. Sci.: Nano*, 2017, **4**, 1283–1293, DOI: **10.1039/c7en00119c**.

98 R. J. Najmanovich, Evolutionary studies of ligand binding sites in proteins, *Curr. Opin. Struct. Biol.*, 2017, **45**, 85–90.

99 B. Kuhn, J. E. Fuchs, M. Reutlinger, M. Stahl and N. R. Taylor, Rationalizing Tight Ligand Binding through Cooperative Interaction Networks, *J. Chem. Inf. Model.*, 2011, **51**, 3180–3198.

100 P. Hobza and J. Řezáč, Introduction: Noncovalent Interactions, *Chem. Rev.*, 2016, **116**, 4911–4912.

101 R. A. Yokel, Physicochemical properties of engineered nanomaterials that influence their nervous system distribution and effects, *Nanomedicine*, 2016, **12**, 2081–2093.

102 T. X. T. Sayle, F. Caddeo, X. Zhang, T. Sakthivel, S. Das, S. Seal, S. Ptasinska and D. C. Sayle, Structure-Activity Map of Ceria Nanoparticles, Nanocubes, and Mesoporous Architectures, *Chem. Mater.*, 2016, **28**, 7287–7295, DOI: **10.1021/acs.chemmater.6b02536**.

103 I. Trenque, G. C. Magnano, M. A. Bolzinger, L. Roiban, F. Chaput, I. Pitault, S. Briançon, T. Devers, K. Masenelli-Varlot, M. Bugnet and D. Amans, Shape-selective synthesis of nanoceria for degradation of paraoxon as a chemical warfare simulant, *Phys. Chem. Chem. Phys.*, 2019, **21**, 5455–5465.

104 L. Huang, J. Chen, L. Gan, J. Wang and S. Dong, Single-atom nanozymes, *Sci. Adv.*, 2019, **5**(5), eaav5490.

105 W. Wu, L. Huang, E. Wang and S. Dong, Atomic engineering of single-atom nanozymes for enzyme-like catalysis, *Chem. Sci.*, 2020, **11**, 9741–9756.

106 K. Chen, S. Sun, J. Wang and X. D. Zhang, Catalytic nanozymes for central nervous system disease, *Coord. Chem. Rev.*, 2021, **432**, 213751.

107 B. Ginovska, O. Y. Gutiérrez, A. Karkamkar, M.-S. Lee, J. A. Lercher, Y. Liu, S. Raugei, R. Rousseau and W. J. Shaw, Bioinspired Catalyst Design Principles: Progress in Emulating Properties of Enzymes in Synthetic Catalysts, *ACS Catal.*, 2023, **13**, 11883–11901.

108 J. P. Klinman, Dynamically Achieved Active Site Precision in Enzyme Catalysis, *Acc. Chem. Res.*, 2014, **48**, 449–456.

109 C. Andreini, I. Bertini, G. Cavallaro, G. L. Holliday and J. M. Thornton, Metal ions in biological catalysis: From enzyme databases to general principles, *J. Biol. Inorg. Chem.*, 2008, **13**, 1205–1218.

110 P. Janoš, A. Spinello and A. Magistrato, All-atom simulations to studying metallodrugs/target interactions, *Curr. Opin. Chem. Biol.*, 2021, **61**, 1–8.

111 P. Janoš, T. Trnka, S. Kozmon, I. Tvaroška and J. Koča, Different QM/MM Approaches To Elucidate Enzymatic Reactions: Case Study on ppGalNAcT2, *J. Chem. Theory Comput.*, 2016, **12**, 6062–6076.

112 P. Janoš, S. Kozmon, I. Tvaroška and J. Koča, How Mycobacterium tuberculosis Galactofuranosyl Transferase 2 (GlfT2) Generates Alternating β-(1–6) and β-(1–5) Linkages: A QM/MM Molecular Dynamics Study of the Chemical Steps, *Chem. – Eur. J.*, 2018, **24**, 7051–7059.

113 T. Trnka, S. Kozmon, I. Tvaroška and J. Koča, Stepwise Catalytic Mechanism via Short-Lived Intermediate Inferred from Combined QM/MM MERP and PES Calculations on Retaining Glycosyltransferase ppGalNAcT2, *PLoS Comput. Biol.*, 2015, **11**, e1004061.

114 T. Dudev and C. Lim, Metal Binding Affinity and Selectivity in Metalloproteins: Insights from Computational Studies, *Annu. Rev. Biophys.*, 2008, **37**, 97–116.

115 J. C. Védrine, Revisiting active sites in heterogeneous catalysis: Their structure and their dynamic behaviour, *Appl. Catal., A*, 2014, **474**, 40–50.

116 H. Ma, E. S. Sollund, W. Zhang, E. Fenes, Y. Qi, Y. Wang, K. R. Rout, T. Fuglerud, M. Piccinini and D. Chen, Kinetic modeling of dynamic changing active sites in a Mars-van Krevelen type reaction: Ethylene oxychlorination on K-doped $CuCl_2/Al_2O_3$, *Chem. Eng. J.*, 2021, **407**, 1385–8947.

117 G. Zhou, W. Geng, L. Sun, X. Wang, W. Xiao, J. Wang and L. Wang, Influence of Mixed Valence on the Formation of Oxygen Vacancy in Cerium Oxides, *Materials*, 2019, **12**, 4041.

118 C. Loschen, A. Migani, S. T. Bromley, F. Illas and K. M. Neyman, Density functional studies of model cerium oxide nanoparticles, *Phys. Chem. Chem. Phys.*, 2008, **10**, 5730–5738.

119 A. Migani, G. N. Vayssilov, S. T. Bromley, F. Illas and K. M. Neyman, Dramatic reduction of the oxygen vacancy formation energy in ceria particles: A possible key to their remarkable reactivity at the nanoscale, *J. Mater. Chem.*, 2010, **20**, 10535–10546.

120 R. Farra, M. García-Melchor, M. Eichelbaum, M. Hashagen, W. Frandsen, J. Allan, F. Girgsdies, L. Szentmiklósi, N. López and D. Teschner, Promoted ceria: A structural, catalytic, and computational study, *ACS Catal.*, 2013, **3**, 2256–2268.

121 X. Han, N. Amrane, Z. Zhang and M. Benkraouda, Unraveling the Negative Role of Oxygen-Vacancy Cluster in Ionic Conductivity in CeO2: Hybrid Functional Study, *J. Phys. Chem. C*, 2018, **122**, 5871–5880.

122 L. Sun, X. Huang, L. Wang and A. Janotti, Disentangling the role of small polarons and oxygen vacancies in Ce O2, *Phys. Rev. B*, 2017, **95**, 245101.

123 M. Nolan, J. E. Fearon and G. W. Watson, Oxygen vacancy formation and migration in ceria, *Solid State Ionics*, 2006, **177**, 3069–3074.

124 F. Xu, Q. Lu, P. J. J. Huang and J. Liu, Nanoceria as a DNase i mimicking nanozyme, *Chem. Commun.*, 2019, **55**, 13215–13218.

125 X. Li, G. Yang, W. Wu and G. Tu, Study on Roast Reaction Kinetics and Crystal Behavior of Ceria-Based Rare Earth Polishing Powder, *J. Rare Earths*, 2007, **25**, 134–138.

126 A. Dannenberg and S. O. Pehkonen, Investigation of the Heterogeneously Catalyzed Hydrolysis of Organophosphorus Pesticides, *J. Agric. Food Chem.*, 1998, **46**, 325–334.

127 F. Esch, S. Fabris, L. Zhou, T. Montini, C. Africh, P. Fornasiero, G. Comelli and R. Rosei, Chemistry: Electron localization determines defect formation on ceria substrates, *Science*, 2005, **309**, 752–755.

128 G. Pacchioni, Oxygen Vacancy: The Invisible Agent on Oxide Surfaces, *ChemPhysChem*, 2003, **4**, 1041–1047.

129 L. Wang, Y. Yu, H. He, Y. Zhang, X. Qin and B. Wang, Oxygen vacancy clusters essential for the catalytic activity of CeO_2 nanocubes for o-xylene oxidation, *Sci. Rep.*, 2017, **7**, 12845.

130 G. Zhuang, Y. Chen, Z. Zhuang, Y. Yu and J. Yu, *Sci. China Mater.*, 2020, **63**, 2089–2118.

131 C. Zhang, A. Michaelides, D. A. King and S. J. Jenkins, Oxygen vacancy clusters on ceria: Decisive role of cerium f electrons, *Phys. Rev. B: Condens. Matter Mater. Phys.*, 2009, **79**, 1–11.

132 L. Wang, Y. Yu, H. He, Y. Zhang, X. Qin and B. Wang, Oxygen vacancy clusters essential for the catalytic activity of CeO2 nanocubes for o-xylene oxidation, *Sci. Rep.*, 2017, **7**, 12845, DOI: **10.1038/s41598-017-13178-6**.

133 M. Nolan, J. E. Fearon and G. W. Watson, Oxygen vacancy formation and migration in ceria, *Solid State Ionics*, 2006, **177**, 3069–3074.

134 C. Gunawan, M. S. Lord, E. Lovell, R. J. Wong, M. S. Jung, D. Oscar, R. Mann and R. Amal, Oxygen-Vacancy Engineering of Cerium-Oxide Nanoparticles for Antioxidant Activity, *ACS Omega*, 2019, **4**, 9473–9479.

135 J. Varalda, C. A. Dartora, P. C. de Camargo, A. J. A. de Oliveira and D. H. Mosca, Oxygen diffusion and vacancy migration thermally-activated govern high-temperature magnetism in ceria, *Sci. Rep.*, 2019, **9**, 4708.

136 F. Zhang, P. Wang, J. Koberstein, S. Khalid and S.-W. Chan, Cerium oxidation state in ceria nanoparticles studied with X-ray photoelectron spectroscopy and absorption near edge spectroscopy, *Surf. Sci.*, 2004, **563**, 74–82.

137 Y.-H. Kim, S.-K. Kim, N. Kim, J.-G. Park and U. Paik, Crystalline structure of ceria particles controlled by the oxygen partial pressure and STI CMP performances, *Ultramicroscopy*, 2008, **108**, 1292–1296.

138 Y. Ma, W. Gao, Z. Zhang, S. Zhang, Z. Tian, Y. Liu, J. C. Ho and Y. Qu, Regulating the surface of nanoceria and its applications in heterogeneous catalysis, *Surf. Sci. Rep.*, 2018, **73**, 1–36.

139 K. Korschelt, R. Schwidetzky, F. Pfitzner, J. Strugatchi, C. Schilling, M. von der Au, K. Kirchhoff, M. Panthöfer, I. Lieberwirth, M. N. Tahir, C. Hess, B. Meermann and W. Tremel, CeO $_{2-x}$ nanorods with intrinsic urease-like activity, *Nanoscale*, 2018, **10**, 13074–13082.

140 E. Paparazzo, On the number, binding energies, and mutual intensities of Ce3d peaks in the XPS analysis of cerium oxide systems: A response to Murugan et al., Superall Microstruct. 85 (2015) 321, *Superlattices Microstruct.*, 2016, **105**, 216–220.

141 A. Allahgholi, J. I. Flege, S. Thieß, W. Drube and J. Falta, Oxidation-State Analysis of Ceria by X-ray Photoelectron Spectroscopy, *ChemPhysChem*, 2015, **16**, 1083–1091.

142 C. Sims, R. Maier, A. Johnston-Peck, J. Gorham, V. A. Hackley and B. Nelson, Approaches for the quantitative analysis of oxidation state in cerium oxide nanomaterials, *Nanotechnology*, 2018, **30**, 085703, DOI: **10.1088/1361-6528/aae364**.

143 J. J. Plata, A. M. Márquez and J. F. Sanz, Transport properties in the CeO_{2-x}(111) surface: From charge distribution to ion-electron collaborative migration, *J. Phys. Chem. C*, 2013, **117**, 25497–25503.

144 M. J. Manto, P. Xie and C. Wang, Catalytic Dephosphorylation Using Ceria Nanocrystals, *ACS Catal.*, 2017, **7**, 1931–1938.

145 R. M. Daniel, M. J. Danson, R. Eisenthal, C. K. Lee and M. E. Peterson, The effect of temperature on enzyme activity: New insights and their implications, *Extremophiles*, 2008, **12**, 51–59.

146 V. Stepankova, S. Bidmanova, T. Koudelakova, Z. Prokop, R. Chaloupkova and J. Damborsky, Strategies for Stabilization of Enzymes in Organic Solvents, *ACS Catal.*, 2013, **3**, 2823–2836.

147 F. van der Ent, S. Skagseth, B. A. Lund, J. Sočan, J. J. Griese, B. O. Brandsdal and J. Åqvist, Computational design of the temperature optimum of an enzyme reaction, *Sci. Adv.*, 2023, **9**, eadi0963.

148 I. Safarik, K. Pospiskova, E. Baldikova and M. Safarikova, Magnetically responsive biological materials and their applications, *Adv. Mater. Lett.*, 2016, **7**, 254–261.

149 M. M. Pedroso, D. Hine, S. Hahn, W. M. Chmielewicz, J. Diegel, L. Gahan and G. Schenk, Pesticide degradation by immobilised metalloenzymes provides an attractive avenue for bioremediation, *EFB Bioeconomy J.*, 2021, **1**, 100015.

150 H. T. Bui, S. Weon, J. W. Bae, E. J. Kim, B. Kim, Y. Y. Ahn, K. Kim, H. Lee and W. Kim, Oxygen vacancy engineering of cerium oxide for the selective photocatalytic oxidation of aromatic pollutants, *J. Hazard. Mater.*, 2021, **404**, 123976.

151 J. Xu and C. Zhang, Oxygen vacancy engineering on cerium oxide nanowires for room-temperature linalool detection in rice aging, *J. Adv. Ceram.*, 2022, **11**, 1559–1570.

152 P. Janos, P. Kuran, V. Pilarova, J. Trogl, M. Stastny, O. Pelant, J. Henych, S. Bakardjieva, O. Zivotsky, M. Kormunda, K. Mazanec and M. Skoumal, Magnetically separable reactive sorbent based on the CeO_2/gamma-Fe_2O_3 composite and its utilization for rapid degradation of the organophosphate pesticide parathion methyl and certain nerve agents, *Chem. Eng. J.*, 2015, **262**, 747–755.

153 J. Henych, P. Ryšánek, M. Šťastný, Z. Němečková, S. Adamec, M. Kormunda, S. Kamínková, K. Hamalová, J. Tolasz and P. Janoš, Electrospun PA6 Nanofibers Bearing the CeO2 Dephosphorylation Catalyst, *ACS Omega*, 2023, **8**, 26610–26618, DOI: **10.1021/acsomega.3c03561**.

154 J. Henych, M. Šťastný, Z. Němečková, K. Mazanec, J. Tolasz, M. Kormunda, J. Ederer and P. Janoš, Bifunctional TiO_2/CeO_2 reactive adsorbent/photocatalyst for degradation of bis-p-nitrophenyl phosphate and CWAs, *Chem. Eng. J.*, 2021, **414**, 128822, DOI: **10.1016/j.cej.2021.128822**.

155 J. A. Brannigan and A. J. Wilkinson, Protein engineering 20 years on, *Nat. Rev. Mol. Cell Biol.*, 2002, **3**, 964–970.

156 D. Hilvert, Design of Protein Catalysts, *Annu. Rev. Biochem.*, 2013, **82**, 447–470.

157 C. Zeymer and D. Hilvert, Directed Evolution of Protein Catalysts, *Annu. Rev. Biochem.*, 2018, **87**, 131–157.

158 Y. Wang, P. Xue, M. Cao, T. Yu, S. T. Lane and H. Zhao, Directed Evolution: Methodologies and Applications, *Chem. Rev.*, 2021, **121**, 12384–12444.

159 C. J. Wilson, Rational protein design: developing next-generation biological therapeutics and nanobiotechnological tools, *Wiley Interdiscip. Rev.: Nanomed. Nanobiotechnol.*, 2015, **7**, 330–341.

160 F. Peccati, S. Alunno-Rufini and G. Jiménez-Osés, Accurate Prediction of Enzyme Thermostabilization with Rosetta Using AlphaFold Ensembles, *J. Chem. Inf. Model.*, 2023, **63**, 898–909.

161 Q. Chen, Y. Xiao, E. I. Shakhnovich, W. Zhang and W. Mu, Semi-rational design and molecular dynamics simulations study of the thermostability enhancement of cellobiose 2-epimerases, *Int. J. Biol. Macromol.*, 2020, **154**, 1356–1365.

162 S. Lutz, Beyond directed evolution—semi-rational protein engineering and design, *Curr. Opin. Biotechnol.*, 2010, **21**, 734–743.

163 M. Reetz, Making Enzymes Suitable for Organic Chemistry by Rational Protein Design, *ChemBioChem*, 2022, **23**, e202200049.

164 P. Xu, Z. F. Ni, M. H. Zong, X. Y. Ou, J. G. Yang and W. Y. Lou, Improving the thermostability and activity of Paenibacillus pasadenensis chitinase through semi-rational design, *Int. J. Biol. Macromol.*, 2020, **150**, 9–15.

165 M. A. Smith, P. A. Romero, T. Wu, E. M. Brustad and F. H. Arnold, Chimeragenesis of distantly-related proteins by noncontiguous recombination, *Protein Sci.*, 2013, **22**, 231–238.

166 V. V. Shende, N. R. Harris, J. N. Sanders, S. A. Newmister, Y. Khatri, M. Movassaghi, K. N. Houk and D. H. Sherman, Molecular Dynamics Simulations Guide Chimeragenesis and Engineered Control of Chemoselectivity in Diketopiperazine Dimerases, *Angew. Chem., Int. Ed.*, 2023, **62**, e202210254, DOI: **10.1002/anie.202210254**.

167 D. Hilvert, Critical Analysis of Antibody Catalysis, *Annu. Rev. Biochem.*, 2003, **69**, 751–793.

168 M. M. Mader and P. A. Bartlett, Binding Energy and Catalysis: The Implications for Transition-State Analogs and Catalytic Antibodies, *Chem. Rev.*, 1997, **97**, 1281–1302.

169 S. Padiolleau-Lefèvre, R. Ben Naya, M. A. Shahsavarian, A. Friboulet and B. Avalle, Catalytic antibodies and their applications in biotechnology: State of the art, *Biotechnol. Lett.*, 2014, **36**, 1369–1379.

170 J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, A. Bridgland, C. Meyer, S. A. A. Kohl, A. J. Ballard, A. Cowie, B. Romera-Paredes, S. Nikolov, R. Jain, J. Adler, T. Back, S. Petersen, D. Reiman, E. Clancy, M. Zielinski, M. Steinegger, M. Pacholska, T. Berghammer, S. Bodenstein, D. Silver, O. Vinyals, A. W. Senior, K. Kavukcuoglu, P. Kohli and D. Hassabis, Highly accurate protein structure prediction with AlphaFold, *Nature*, 2021, **596**, 583–589.

171 M. Baek, F. DiMaio, I. Anishchenko, J. Dauparas, S. Ovchinnikov, G. R. Lee, J. Wang, Q. Cong, L. N. Kinch, R. Dustin Schaeffer, C. Millán, H. Park, C. Adams, C. R. Glassman, A. DeGiovanni, J. H. Pereira, A. V. Rodrigues, A. A. Van Dijk, A. C. Ebrecht, D. J. Opperman, T. Sagmeister, C. Buhlheller, T. Pavkov-Keller, M. K. Rathinaswamy, U. Dalwadi, C. K. Yip, J. E. Burke, K. Christopher Garcia, N. V. Grishin, P. D. Adams, R. J. Read and D. Baker, Accurate prediction of protein structures and interactions using a three-track neural network, *Science*, 2021, **373**, 871–876.

172 Y. Li, X. Zhang and J. Liu, *Preparing selewctive nanozymes by molecular imprinting*, ed. A. Martín-Esteban, Springer US, New York, NY, 2021, pp. 223–232.

173 R. Wang, J. Pan, M. Qin and T. Guo, Molecularly imprinted nanocapsule mimicking phosphotriesterase for the catalytic hydrolysis of organophosphorus pesticides, *Eur. Polym. J.*, 2019, **110**, 1–8.

174 J. Pan, S. Liu, H. Jia, J. Yang, M. Qin, T. Zhou, Z. Chen, X. Jia and T. Guo, Rapid hydrolysis of nerve agent simulants by molecularly imprinted porous crosslinked polymer incorporating mononuclear zinc(II)-picolinamine-amidoxime module, *J. Catal.*, 2019, **380**, 83–90, DOI: **10.1016/j.jcat.2019.10.023**.

175 R. MacCarone, A. Tisi, M. Passacantando and M. Ciancaglini, Ophthalmic Applications of Cerium Oxide Nanoparticles, *J. Ocul. Pharmacol. Ther.*, 2020, **36**, 376–383.

176 H. Sadidi, S. Hooshmand, A. Ahmadabadi, S. J. Hoseini, F. Baino, M. Vatanpour and S. Kargozar, Cerium oxide nanoparticles (nanoceria): Hopes in tissue engineering, *Molecules*, 2020, **25**, 4559.

177 H. Li, Z.-Y. Yang, C. Liu, Y.-P. Zeng, Y.-H. Hao, Y. Gu, W.-D. Wang and R. Li, PEGylated ceria nanoparticles used for radioprotection on human liver cells under γ-ray irradiation, *Free Radical Biol. Med.*, 2015, **87**, 26–35.

178 P. Li, S. Y. Moon, M. A. Guelta, S. P. Harvey, J. T. Hupp and O. K. Farha, Encapsulation of a Nerve Agent Detoxifying Enzyme by a Mesoporous Zirconium Metal-Organic Framework Engenders Thermal and Long-Term Stability, *J. Am. Chem. Soc.*, 2016, **138**, 8052–8055, DOI: **10.1021/jacs.6b03673**.

179 X. Hu, X. Wang, Q. Liu, J. Wu, H. Zhang, A. Khalique, Z. Sun, R. Chen, J. Wei, H. Li, D. Kong, J. Zhuang, X. Yan and X. Huang, Nanozyme-Powered Giant Unilamellar Vesicles for Mimicry and Modulation of Intracellular Oxidative Stress, *ACS Appl. Mater. Interfaces*, 2021, **13**, 21087–21096.

180 R. Nishiyabu, N. Hashimoto, T. Cho, K. Watanabe, T. Yasunaga, A. Endo, K. Kaneko, T. Niidome, M. Murata, C. Adachi, Y. Katayama, M. Hashizume and N. Kimizuka, Nanoparticles of adaptive supramolecular networks self-assembled from nucleotides and lanthanide ions, *J. Am. Chem. Soc.*, 2009, **131**, 2151–2158.

181 A. J. Patil, R. Krishna Kumar, N. J. Barron and S. Mann, Cerium oxide nanoparticle-mediated self-assembly of hybrid supramolecular hydrogels, *Chem. Commun.*, 2012, **48**, 7934–7936.

182 J. Han, H. Gong, X. Ren and X. Yan, Supramolecular nanozymes based on peptide self-assembly for biomimetic catalysis, *Nano Today*, 2021, **41**, 101295.

183 S. Abedanzadeh, Z. Moosavi-Movahedi, N. Sheibani and A. A. Moosavi-Movahedi, Nanozymes: Supramolecular perspective, *Biochem. Eng. J.*, 2022, **183**, 108463.

184 T. Heinisch and T. R. Ward, Design strategies for the creation of artificial metalloenzymes, *Curr. Opin. Chem. Biol.*, 2010, **14**, 184–199.

185 C. Mayer, D. G. Gillingham, T. R. Ward and D. Hilvert, An artificial metalloenzyme for olefin metathesis, *Chem. Commun.*, 2011, **47**, 12068–12070.

186 J. Henych, M. Šťastný, S. Kříženecká, J. Čundrle, J. Tolasz, T. Dušková, M. Kormunda, J. Ederer, Š. Stehlík, P. Ryšánek, V. Neubertová and P. Janoš, Ceria-Catalyzed Hydrolytic Cleavage of Sulfonamides, *Inorg. Chem.*, 2024, **63**, 2298–2309.

187 L. M. F. Bertoline, A. N. Lima, J. E. Krieger and S. K. Teixeira, Before and after AlphaFold2: An overview of

protein structure prediction, *Front. Bioinform.*, 2023, **3**, 1120370.
188 G. Casadevall, C. Duran and S. Osuna, AlphaFold2 and Deep Learning for Elucidating Enzyme Conformational Flexibility and Its Application for Design, *JACS Au*, 2023, **3**, 1554–1562.
189 Y. Ming, W. Wang, R. Yin, M. Zeng, L. Tang, S. Tang and M. Li, A review of enzyme design in catalytic stability by artificial intelligence, *Briefings Bioinf.*, 2023, **24**, bbad065, DOI: **10.1093/BIB/BBAD065**.
190 J. Yang, F.-Z. Li and F. H. Arnold, Opportunities and Challenges for Machine Learning-Assisted Enzyme Engineering, *ACS Cent. Sci.*, 2024, **10**, 226–241, DOI: **10.1021/acscentsci.3c01275**.
191 A. Merchant, S. Batzner, S. S. Schoenholz, M. Aykol, G. Cheon and E. D. Cubuk, Scaling deep learning for materials discovery, *Nature*, 2023, **624**, 80–85.
192 N. J. Szymanski, B. Rendy, Y. Fei, R. E. Kumar, T. He, D. Milsted, M. J. McDermott, M. Gallant, E. D. Cubuk, A. Merchant, H. Kim, A. Jain, C. J. Bartel, K. Persson, Y. Zeng and G. Ceder, An autonomous laboratory for the accelerated synthesis of novel materials, *Nature*, 2023, **624**, 86–91.
193 P. L. Kang, C. Shang and Z. P. Liu, Large-Scale Atomic Simulation via Machine Learning Potentials Constructed by Global Potential Energy Surface Exploration, *Acc. Chem. Res.*, 2020, **53**, 2119–2129.
194 S. N. Steinmann, Q. Wang and Z. W. Seh, How machine learning can accelerate electrocatalysis discovery and optimization, *Mater. Horiz.*, 2023, **10**, 393–406.
195 V. L. Deringer, M. A. Caro and G. Csányi, Machine Learning Interatomic Potentials as Emerging Tools for Materials Science, *Adv. Mater.*, 2019, **31**, 1902765.