

Surface Science of Nanozymes and Defining a Nanozyme Unit

Mohamad Zandieh and Juewen Liu*

Department of Chemistry, Waterloo Institute for Nanotechnology, Waterloo, Ontario, N2L 3G1,
Canada

Email: liujw@uwaterloo.ca

Abstract

Nanozymes aim to use nanomaterials to replace protein-based enzymes. Nanozymes have attracted extensive interest due to their stability, cost-effectiveness and versatility. While the focus of the nanozyme field has been mainly on the discovery of new nanozyme materials and exploration of their analytical, biomedical and environmental applications, fundamental studies are growing. Nanozymes are related to two important fields: enzymes and heterogeneous catalysis. Although fitting nanozyme kinetic data to the Michaelis–Menten kinetics is a standard practice, using the surface science methods of heterogeneous catalysis can provide insights about their catalytic mechanism. Definition of a nanozyme unit is critical for understanding and comparing nanozyme activities. In this perspective, we articulate the use of surface science approach to study nanozymes, and discuss the various application scenarios of using different nanozyme units.

Introduction

The development of surface science has a lot to do with heterogeneous catalysis. A hallmark example is the study of the catalytic mechanism of ammonia synthesis using the Haber-Bosch process, where iron-based catalysts were explored.¹ A powerful method is to dissect a reaction in elementary steps on single-crystal surfaces and study each step separately, so-called surface science approach.² The catalytic mechanism is very complex and difficult to prove even for such a seemingly simple reaction (Figure 1A).

Using inorganic catalysts to convert enzyme substrates such as glucose, TMB (3,3',5,5'-tetramethylbenzidine) and DNA, to enzymatic reaction products (e.g. gluconic acid plus H₂O₂, oxidized TMB, and cleaved DNA, respectively) have been extensively studied since the discovery of the peroxidase-like activity of Fe₃O₄ nanoparticles.³ Such nanoparticles are called peroxidase nanozymes since the same products as horseradish peroxidase (HRP) were generated, although they likely have distinct reaction mechanisms.^{4, 5} Based on the current status of the field, nanozymes are defined by their substrates and products, instead of structural or mechanistic features.⁴ With obvious advantages in cost and stability, nanozymes have been used to replace enzymes for therapeutic applications, designing bioanalytical sensors, and degradation of environmental pollutants.⁶⁻⁸

In a typical enzyme catalytic cycle, the reaction steps include substrate binding (often specific), catalytic reaction, and product release (Figure 1B). For nanozymes, the first step is replaced by substrate adsorption (mostly nonspecific), followed by surface reaction and finally product desorption to regenerate surface sites (Figure 1C). From the standpoint of heterogeneous catalysis, there is rich surface science to be learned for nanozymes. Only when reaction mechanisms are fully elucidated, can one draw mechanistic comparisons with the corresponding enzymes and to perform rational design to better mimic enzymes. For example, specificity and high catalytic efficiency are still challenging for nanozymes.⁹

So far, the nanozyme field has mainly focused on identification of new and better nanozyme materials, expansion of reaction types, test of new substrates, and push for applications.⁶⁻⁸ Yet, surface science research of nanozyme reactions has been limited. A major challenge is that nanozymes work in the solution phase under physiologically relevant conditions. Thus, the standard ultrahigh vacuum techniques cannot be used. In addition, enzyme substrates are

typically more complex compared to simple gas molecules, also making mechanistic studies more difficult. Finally, nanomaterials cannot be simply substituted by single crystal surfaces for study since the catalytic activity is often a function of particle size. Nevertheless, efforts have been made to elucidate nanozyme catalytic mechanisms,¹⁰ and some surface science study examples are described below.

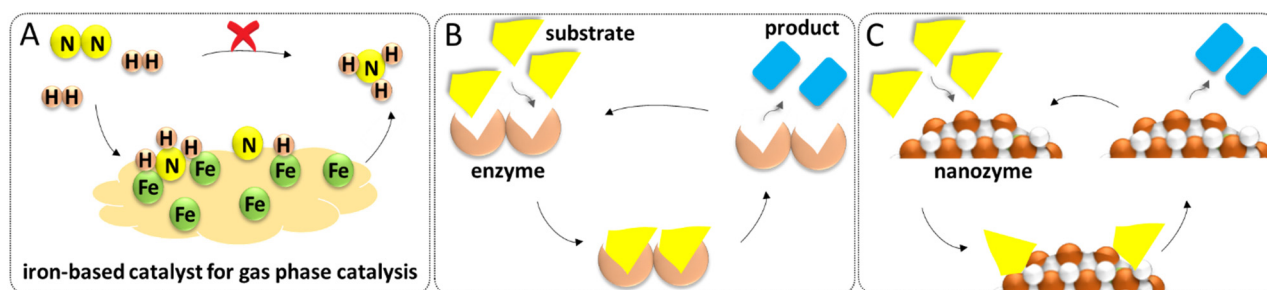


Figure 1. Schemes showing (A) typical heterogeneous catalysis with gas phase substrates such as N_2 and H_2 at high temperature and pressure, (B) enzyme catalysis under ambient conditions, and (C) nanozyme catalysis under ambient conditions. Nanozymes and enzymes have the same substrates and products, yet the reaction process is closer to heterogeneous catalysis.

Metal Oxides as a Peroxidase Nanozyme. Fe_3O_4 nanoparticles is one of the most studied peroxidase nanozymes. Recently, Peng and coworkers investigated the origin of peroxidase-like activity of Fe_3O_4 nanoparticles and other transition metal oxides by dissecting the surface reactions into the following steps including: hydroxyl radical ($\text{OH}\cdot$) and hydroperoxyl radical ($\text{HO}_2\cdot$) generation, chromogenic substrate oxidation, and surface active site regeneration (Figure 2A).⁵ Employing techniques such as H_2 -temperature programmed reduction, O_2 -temperature programmed oxidation, and electron paramagnetic resonance (EPR) spectroscopy, it was discovered that the redox reaction between the surface metal cation (oxidation) and H_2O_2 (reduction) was the rate limiting step for the catalytic activity. The $\text{OH}\cdot$ radicals generated in this step oxidize not only the chromogenic substrate but also another H_2O_2 molecule to provide $\text{HO}_2\cdot$ radicals for regeneration of the surface active sites (reduction of M^{n+1} back to M^{n+}). Using solid experimental evidence, this surface science study articulated the mechanistic difference between nanozymes and HRP. For the latter, radicals stay on the Fe-protoporphyrin ring. Similar surface

science work was also performed on single-atom nanozymes, where the $\text{OH}\cdot$ -mediated pathway was identified as well.¹¹

CeO₂ as an Oxidase Nanozyme. CeO₂ nanoparticles can catalyze the oxidation of a variety of chromogenic substrates such as TMB and ABTS (2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), and a generally accepted mechanism is the activation of oxygen by the oxygen vacancies on CeO₂ surface.¹² Each oxygen vacancy is accompanied with two Ce^{3+} sites to retain the charge balance. However, by using X-ray spectroscopy, Cafun et al. proposed that each CeO₂ nanoparticle acts as an electron sponge to collectively attract electrons instead of relying on individual Ce^{3+} sites.¹³

Our group examined the steps of substrate adsorption and product release.¹⁴ Using ABTS substrate as an example, it was found that the oxidase-like activity of CeO₂ nanoparticles was directed by electrostatic interactions. Negatively charged ABTS was adsorbed on the surface of CeO₂. After the oxidation reaction, the oxidized ABTS remained adsorbed, inhibiting the adsorption of new substrates, which can be observed from the green color of precipitated CeO₂ nanoparticles (Figure 2B).¹⁵ In the presence of F^- , the surface charge of CeO₂ reversed from positive to negative, indicating capping of F^- on the surface.¹⁴ The surface of the F^- -capped CeO₂ had no color, indicating product desorption to facilitate catalytic turnover (Figure 2C).

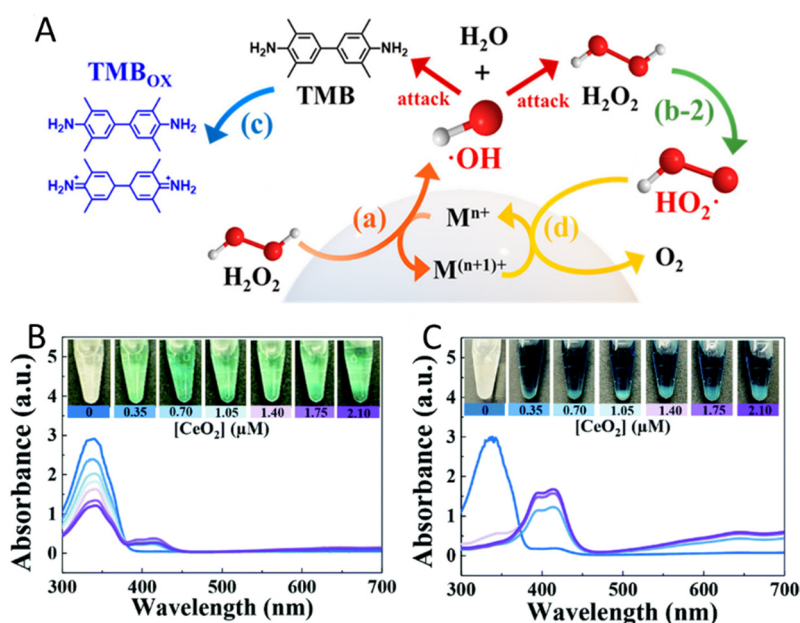


Figure 2. (A) A scheme illustrating the different surface reactions occurring during the peroxidase-like activity of transition metal oxide nanozymes. Photographs and UV-vis spectra (after centrifugation) demonstrating ABTS oxidation by (B) bare CeO₂ nanoparticles where the activity was inhibited by the adsorption of oxidized ABTS, and (C) F⁻-capped nanoparticles where F⁻ facilitated product desorption and enhanced the catalytic turnover. (A) Reprinted with permission.⁵ Copyright 2021, American Chemical Society. (B, C) Reprinted with permission.¹⁵ Copyright 2019, Royal Society of Chemistry.

Construction of substrate binding pockets. A key problem of nanozymes is that they lack a substrate binding pocket, and most substrates are nonspecifically adsorbed leading to poor specificity. Our group solved this problem by coating nanozymes with a layer of molecularly imprinted polymers (MIPs). A substrate molecule such as TMB is adsorbed on a nanozyme (e.g. Fe₃O₄) and an optimal MIP layer is formed. The resulting nanozyme can achieve nearly 100-fold improved specificity (Figure 3A).¹⁶ After a careful study of substrate adsorption, the rate of catalysis was found to be increased likely due to enriched substrate concentration in the MIP layers.¹⁷ The selective adsorption of ABTS over TMB by the ABTS-imprinted Fe₃O₄ (A-MIP) was 3.6-fold of the non-imprinted Fe₃O₄ nanoparticles. When a cationic monomer was incorporated in the MIP layer (A-MIPpos) the selective adsorption increased to 8.5-fold (Figure 3B). Similarly, the activation energy (E_a) of the catalytic reaction was also decreased 2.1- and 1.4-fold for A-MIPpos and A-MIP, respectively, compared to the bare Fe₃O₄ nanoparticles (Figure 3C).

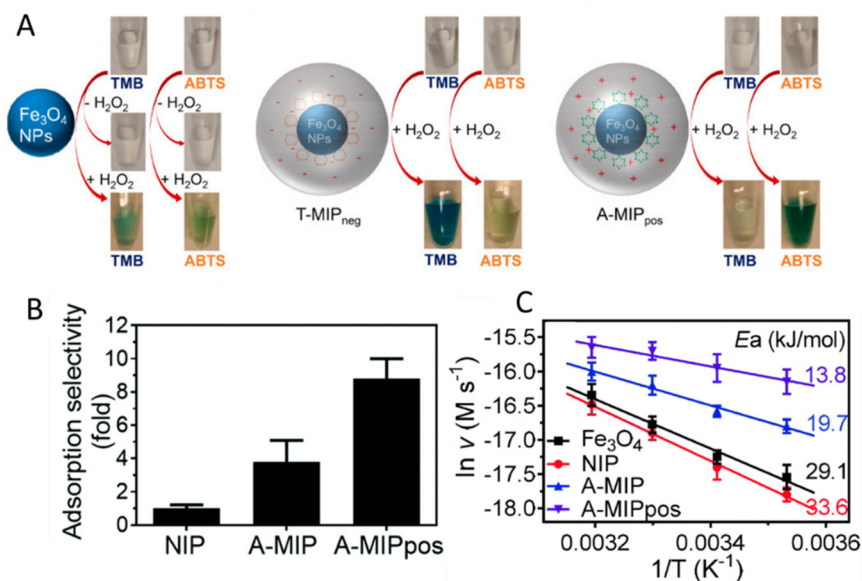


Figure 3. (A) Schemes and photographs illustrating the activity and specificity of bare Fe_3O_4 and MIP-coated Fe_3O_4 nanozymes for peroxidation of TMB and ABTS. (B) Selective adsorption of ABTS over TMB by nanozymes with various MIP layers. (C) Activation energy of various nanozymes for ABTS peroxidation. (A) Reprinted with permission.¹⁶ Copyright 2017, American Chemical Society. (B, C) Reprinted with permission.¹⁷ Copyright 2019, Royal Society of Chemistry.

Computer Simulation. Aside from experimental work, computer simulation could be a highly valuable complementary tool. For example, Shen et al. performed density functional calculations on iron oxide nanosurfaces with different iron oxidation states, chemical compositions, crystal structures, and exposed facets to investigate the atomic-level mechanisms of the peroxidase-like activity.¹⁸ A mechanism was suggested including chemisorption of H_2O_2 on the surface to form two $\text{OH}^\bullet$ radicals, followed by consecutive reduction of two hydroxyl group to oxidize TMB and recover the metal oxide surface. The authors systematically predicted the peroxidase-like activity of various kinds of nanomaterials, and the predictions agreed with reported experimental studies, although the activity of some predicted nanomaterials still need to be confirmed experimentally. The same group later systematically changed the linker substituent in some metal organic framework (MOF) nanozymes to mimic engineered natural enzymes.¹⁹ It was found that ligands with higher electron-drawing (higher Hammett value) promoted a more efficient oxidase-like

activity in such MOF nanozymes, which was confirmed with experimental results. Such simulation studies to predict quantitative relationships between nanozyme surface structure and their catalytic activity would facilitate the design of more effective nanozymes and rationalize their activities.

Definition of a Nanozyme Unit. An enzyme unit is defined by the activity of the enzyme (e.g., the amount of enzyme needed to convert 1 μmol or 1 nmol of substrate in 1 min). So, an enzyme unit is an activity unit. When comparing nanozymes with enzymes or comparing different nanozymes, we need to define a nanozyme unit, and here a nanozyme unit is a physical unit. A clear understanding on this is critical and fundamental for surface science studies of nanozymes.

Each enzyme molecule is typically counted as one active site for catalytic turnover calculations. Although a nanozyme implies the entire nanoparticle, a nanozyme can have more than one active site on the surface. In the literature, surface site, particle, mass, and total metal atom concentrations have all been used to define nanozyme units and calculate or compare the turnover rate (k_{cat}). We reason that these four ways of defining nanozyme units can all be useful depending on application scenarios. 1) To confirm catalytic turnover and perform mechanistic studies from the heterogeneous catalysis standpoint,²⁰ it is important to quantify the number of surface active sites (Figure 4A), which can be done by a range of spectroscopic methods, chemisorption or estimated by geometric calculations.²¹ Most likely, each nanoparticle contains multiple active sites. 2) In most papers, each nanozyme particle is counted as one enzyme unit likely due to its simplicity (Figure 4B). A direct comparison of its k_{cat} with that of an enzyme could result in a mismatch in the number of catalytic sites. Nevertheless, when the volume of a nanozyme and a protein enzyme is comparable, such comparison is also useful (e.g. for preparing enzyme electrodes and studying single nanoparticle catalysis). For example, a HRP protein can be described as $4.0 \times 6.7 \times 11.7 \text{ nm}^3$ based on its crystal structure, which is comparable to the size of many nanoparticles. 3) Using mass concentration is popular for protein enzymes (Figure 4C), since specific enzyme activity is normalized to the mass. Most reactors would rely on mass concentration of enzymes for calculation. Comparisons between enzymes and nanozymes at the same mass concentration were also reported (k_{cat} values cannot be calculated and instead V_{max} values are compared).^{22, 23} 4) Finally, the molar concentration of total metal atoms has been used, such as in the case of single atom nanozymes supported on porous materials (Figure 4D).²⁴ Most of these nanozyme units can be interconverted, although the k_{cat} values would be different. We recommend

researchers to provide all the needed unit conversion information such as nanozyme size, shape, density, concentration, volume and even light pathlength for absorbance measurement. Alternatively, such conversion can be provided in papers. Most importantly, k_{cat} values should not be compared without understanding how its nanozyme unit is defined.

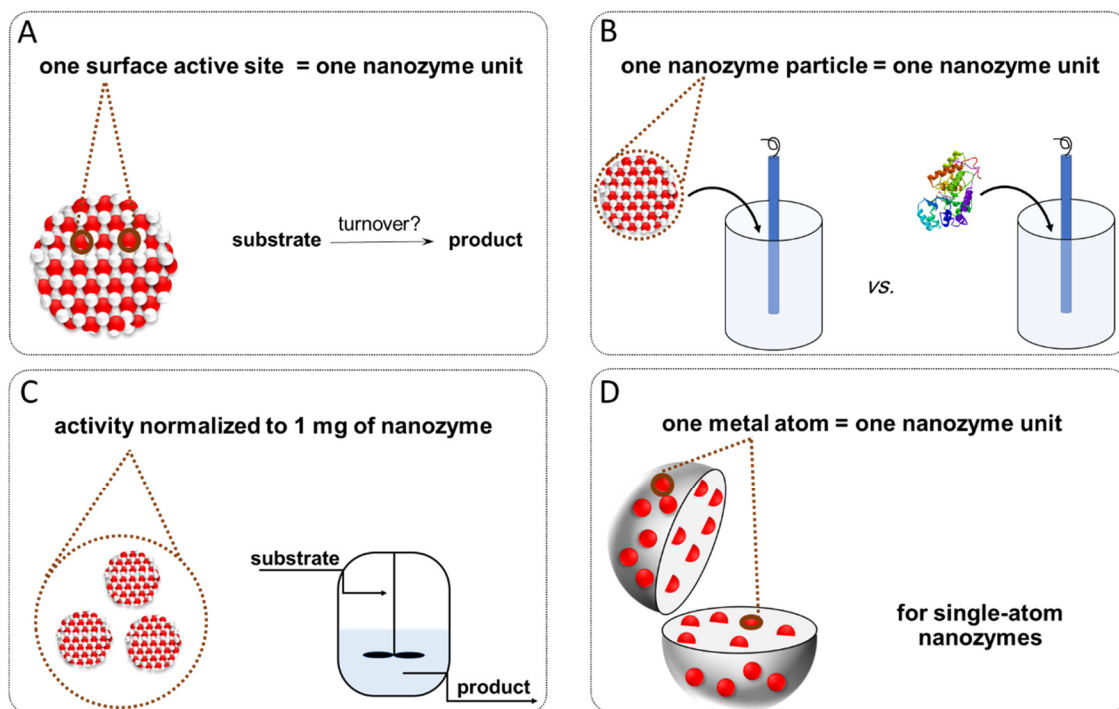


Figure 4. Different definitions of a nanozyme unit based on (A) the molar concentration of surface-active sites, which is useful for confirming catalytic turnover; (B) the molar concentration of nanoparticles, which is useful for applications such as enzyme electrodes; (C) the mass concentration of nanoparticles, which is useful for enzyme reactors; and (D) the molar concentration of total metal atoms for single atom nanozymes.

Nanozymes: homogeneous or heterogeneous catalysts? Chemically, nanozymes are heterogeneous catalysts but they are intended to replace enzymes as homogenous catalysts. In terms of applications and ambient reaction conditions, nanozymes are close to enzymes. In terms of reaction mechanisms and separation of catalysts, nanozymes are close to heterogeneous catalysts. In the nanozyme field, describing nanozyme activity by using the Michaelis–Menten kinetic model has been a widely used practice, implying the use of enzymology methods. The use of $k_{\text{cat}}/K_{\text{m}}$ to compare different enzymes, however, can be misleading.²⁵ This ratio is more useful

for comparing different substrates catalyzed by the same enzyme, instead of comparing different enzymes. k_{cat} is a term of enzymology, whereas in heterogeneous, turnover number or turnover frequency is more often used.²⁶ In this Perspective, we discussed the surface science approach to study nanozymes as heterogeneous catalysts. For fundamental studies, using heterogeneous catalysis methods will likely to be more productive.

Perspectives. In this article, we first discussed the similarities and differences between typical heterogeneous catalysis, enzymes and nanozymes. The need to perform solution phase reactions under ambient conditions plus the use of complex enzyme substrates has made it even more challenging to elucidate the surface science of nanozymes. The chemistry of nanozyme is more related to surface science in terms of reaction mechanism. Just like any heterogeneous catalysts, the steps of substrate binding (adsorption), catalytic reaction, and product release (desorption) are also the key steps in nanozymes. By tuning the charge of substrates, changing pH and ionic strength, and surface modification, the adsorption affinity with nanozymes can be controlled. Challenges are mainly in the catalytic reaction step. By using the experimental methods of heterogeneous catalysis, we believe that the nanozyme field can expect much deeper and quantitative mechanistic understandings. A few studies have already been published on this front. Based on current studies, most nanozymes do not have the same mechanism as their enzyme counterparts, even though they have the same substrates and products. Future studies in the nanozyme surface science field would likely focus on a few model systems to elucidate the elementary steps of the reactions. With such understanding, it might be possible to engineer better nanozymes. For most nanozymes, understanding of substrate adsorption and product desorption can be relatively easily achieved.

The definition of a nanozyme unit needs to be carefully understood and clearly presented in publications. Such standardization efforts will make it productive to link nanozyme work with enzymes and heterogeneous catalysis.

Notes

The authors declare no competing financial interest.

Acknowledgements

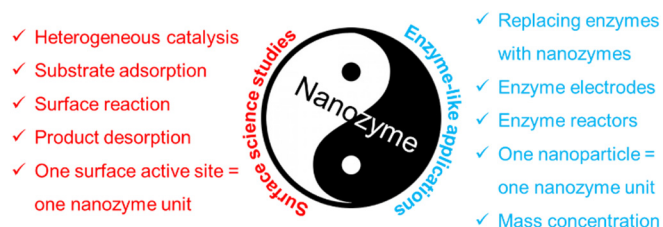
Funding for this work was from the Natural Sciences and Engineering Research Council of Canada (NSERC).

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Author Biography

Mohamad Zandieh received his M.Sc. in Biotechnology Engineering from Sharif University of Technology in Iran (2017). He is currently a Ph.D. student under the supervision of Professor Juewen Liu in the Department of Chemistry at the University of Waterloo. His present research focuses on functional DNA and surface science of nanomaterials.

Prof. Juewen Liu received his Ph.D. degree from the University of Illinois at Urbana-Champaign in 2005. He is currently a professor of chemistry at the University of Waterloo. He serves as a Section Editor for *Biosensors & Bioelectronics* and a Contributing Editor for *Trends in Analytical Chemistry*. He is on the editorial board of *FACETS* and advisory board of *Langmuir*. He is interested in metal-dependent DNAzymes, aptamers, biointerface sciences, and nanozymes. Since 2002, he has published over 400 papers, receiving over 31,000 citations with an H-index of 85.