



Enzyme-Like Properties of Gold Clusters for Biomedical Application

Yunguang Zhang¹, Shuo Li¹, Haile Liu², Wei Long^{3*} and Xiao-Dong Zhang^{2*}

¹ School of Science, Xi'an University of Posts and Telecommunications, Xi'an, China, ² Department of Physics and Tianjin Key Laboratory of Low Dimensional Materials Physics and Preparing Technology, School of Sciences, Tianjin University, Tianjin, China, ³ Institute of Radiation Medicine, Chinese Academy of Medical Sciences and Peking Union Medical College, Tianjin, China

In recent years, the rapid development of nanoscience and technology has provided a new opportunity for the development and preparation of new inorganic enzymes. Nanozyme is a new generation of artificial mimetic enzyme, which like natural enzymes, can efficiently catalyze the substrate of enzyme under mild conditions, exhibiting catalytic efficiency, and enzymatic reaction kinetics similar to natural enzymes. However, nanozymes exist better stability than native enzymes, it can still maintain 85 % catalytic activity in strong acid and alkali (pH 2~10) or large temperature range (4~90°C). This provides conditions for designing complex catalytic systems. In this review, we discussed the enzymatic attributes and biomedical applications of gold nanoclusters, including peroxidase-like, catalase-like, detection of heavy metal ions, and therapy of brain and cancer etc. This review can help us understand the current research status nanozymes.

OPEN ACCESS

Edited by:

Kelong Fan,
Institute of Biophysics (CAS), China

Reviewed by:

Rong Yang,
National Center for Nanoscience and
Technology (CAS), China
Xiangheng Niu,
Jiangsu University, China
Lizeng Gao,
Institute of Biophysics (CAS), China

*Correspondence:

Wei Long
longway@irm-cams.ac.cn
Xiao-Dong Zhang
xiaodongzhang@tju.edu.cn

Specialty section:

This article was submitted to
Nanoscience,
a section of the journal
Frontiers in Chemistry

Received: 03 January 2020

Accepted: 09 March 2020

Published: 03 April 2020

Citation:

Zhang Y, Li S, Liu H, Long W and
Zhang X-D (2020) Enzyme-Like
Properties of Gold Clusters for
Biomedical Application.
Front. Chem. 8:219.
doi: 10.3389/fchem.2020.00219

Keywords: gold nanoclusters, nanozyme, enzyme-like properties, cancer therapy, bio-detection

INTRODUCTION

The natural enzyme is a kind of biocatalyst that is closely related to many life activities (Arnold et al., 2001). Similar to other catalysts, the presence of natural enzymes can greatly speed up the reaction and sometimes participate in the reaction, but the enzyme itself does not change before or after the reactions (Wilhelmová, 1996). In addition, it exhibits high catalytic efficiency, mild reaction conditions and high specificity (Sheldon, 2007). However, lots of natural enzymes are proteins, which are susceptible to high temperature, acidic or alkaline, causing loss of catalytic activity (Chang, 2013). In addition, natural enzymes presented in living organisms are difficult to purify, relatively expensive, and not easily transported and stored (Scopes, 2013). To overcome the limitations of instability and high cost for natural enzymes, researchers are looking for different ways to prepare mimetic enzymes to replace natural enzymes (Chen et al., 2010; Yang et al., 2016). Until now, different types of mimic enzymes have been designed and used in many biological fields, such as immunoassay (Lequin, 2005), glucose detection (Song et al., 2010), heavy metal ion detection (Kim et al., 2001), free radical protection (Barzegar and Moosavi-Movahedi, 2011), and tissue engineering (Griffith and Naughton, 2002). Host-guest chemistry (Wan et al., 2006) and supramolecular chemistry (Steed and Atwood, 2013) lay an important theoretical foundation for mimic enzyme. In essence, the basic meaning of host-guest chemistry comes from the interaction between enzyme and substrate, which is embodied in the complementary spatial and electronic arrangement of the binding site between the subject and the guest (Spichiger-Keller, 2008). This host-guest complementarity is similar to the combination of the nanozymes and the substrate. Based on non-covalent bond interactions, such as electrostatic interactions, hydrogen bonding and van der Waals forces (Pasternack et al., 1998; Wei and Wang, 2013), supramolecules are produced by the combination of

substrate and receptor (Geim and Grigorieva, 2013). When receptors combine with complex ions or molecules, a supramolecule with stable structure and properties is formed, which has the functions of molecular recognition, catalytic and selective output. Host-guest chemistry and supramolecules chemistry are important theoretical weapons for the simulation of artificial enzymes.

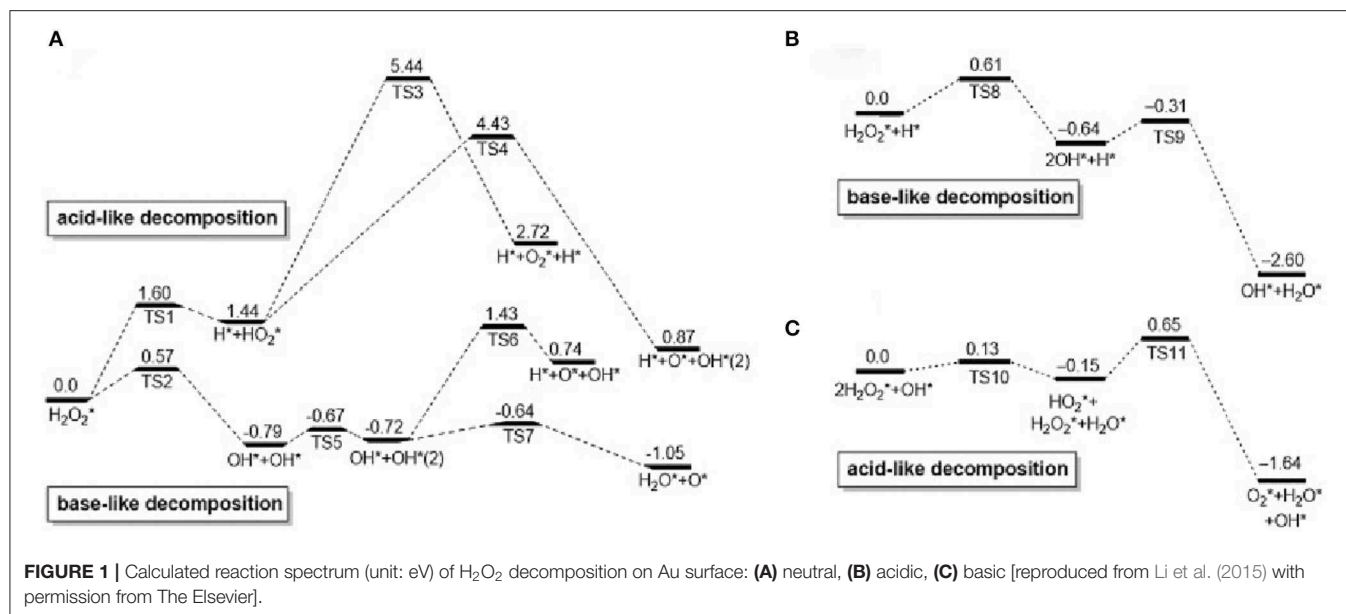
At present, the ideal traditional enzyme systems include cyclodextrin (Del Valle, 2004), cyclophane (Gleiter and Hopf, 2006) and cyclic aromatic hydrocarbons (Tsipis and Tsipis, 2003). The chemical composition of traditional mimic *peroxidase-like activation* enzyme is non-protein, but these enzymes have similar catalytic performance to natural enzymes (Meeuwissen and Reek, 2010). These traditional mimetic enzymes are superior to natural enzymes in thermal stability and acid-base resistance, but their shortcomings, such as too-complex structure, difficulties in separation, single catalytic active sites, and low catalytic efficiency, still stunt their development progress (Liu Q. et al., 2017). With the development of nanomaterials, the researches on the catalysis of nanomaterials have also achieved many new results (Yin and Talapin, 2013). Nanozymes have the dual identities of enzymes and nanomaterials (Roduner, 2006). Unlike natural enzymes or traditional artificial enzymes, nanozymes (Wang et al., 2013; Li et al., 2015; Zhou et al., 2017; Fan et al., 2018; Qu et al., 2018; Chen et al., 2019; Huang et al., 2019a,b; Jiang et al., 2019; Liang and Yan, 2019; Liu et al., 2019; Mu et al., 2019a,b; Wu et al., 2019; Xu et al., 2019; Yan et al., 2019) have many physical and chemical properties, besides their catalytic functions (Senanayake et al., 2013). For example, Fe₃O₄ and CdS nanoparticles not only have the catalytic functions of peroxidase and superparamagnetic activities, but also have the property of luminescence (Liu et al., 2011). The enzymatic activity of nanozymes is not only related to its composition, crystal form and structure, but also to its surface properties (Lin et al., 2014a). Take gold nanoclusters (Au NCs) as an example, the combination of certain small molecules with Au NCs can change the surface microenvironment, resulting in changes in the catalytic activities of Au NCs (Jin, 2010). Wang et al. found that the surface modification of Au NCs can affect its catalytic activity (Yuwen et al., 2014). The Au NCs were modified with amino and citric acid respectively to make positive and negative charges on the surface, and then catalytically oxidize ABTS (Erel, 2004) (negatively charged) and TMB (Ding et al., 2018) (positively charged) to detect its catalytic activity. The amino and citric acid modified Au NCs were found to have high affinity to the substrates ABTS and TMB. Not only small molecules can be combined with Au NCs, inorganic nanoparticles, metal ions and biomacromolecules (such as DNA, RNA) can also be combined with it to change the surface microenvironment of Au NCs, thereby changing its catalytic activity. Compared with other nanomaterials with simulating peroxidase, gold nanoclusters have the advantages of small size, good stability, good biocompatibility, and are more prominent in the application of biological analysis. However, the potential of Au NCs as enzyme mimics is easily limited by the low catalytic activity at neutral environment. In addition, since the surface atom is the key catalytic sites for gold clusters, modification of

nanozymes with various coating molecules may block their active sites, reducing or inhibiting their enzymatic activity. This review details the enzymatic properties of gold nanoclusters and their applications in biomedicine in recent years.

ENZYME-LIKE ACTIVITY OF GOLD CLUSTERS

Peroxidase-Like Property of Gold Clusters

Peroxidase (Gao et al., 2007) is a kind of natural enzyme that have catalytic oxidation effects on hydrogen peroxide. The establishment of an analytical method involving H₂O₂ is of great significance in analytical chemistry and clinical medicine. In recent years, Au NCs have been reported to have peroxidase-like properties and are used in the fields of bionics, biosensing, and biomedicine (Feng et al., 2017; Liao et al., 2017). First, H₂O₂ can be adsorbed on the surface of gold nanomaterials, and the O–O bonds of H₂O₂ may be decomposed into dihydroxy radicals; at the same time, the generated hydroxyl radicals may be stabilized by gold nanomaterials through partial electron exchange interactions. This may contribute to their catalytic capabilities. **Figure 1** shows the adsorption and decomposition of H₂O₂ on Au(111) under different pH conditions (Li et al., 2015). Under neutral conditions, H₂O₂ adsorbed on the surface of Au (111) can undergo acid decomposition and alkali decomposition. According to the principle of lowest energy, alkaline decomposition is more inclined under neutral conditions (**Figure 1A**) (He et al., 2012). It is worth noting that under these conditions, the adsorbed O* cannot generate O₂ under the high energy barrier of 1.42 eV (Wu et al., 2019). Under acidic conditions, the decomposition pathway of H₂O₂ is similar to the decomposition of alkalis under neutral conditions. First, OH* is generated and then O* and H₂O* are generated. The generated O* can extract H from the substrate. Therefore, under acidic and neutral conditions, Au (111) has peroxidase activity (**Figure 1B**). Ding et al. used 3, 3', 5,5'-tetramethylbenzidine (TMB) as a substrate and found that histidine-modified gold clusters (His-Au NCs) have peroxidase-like activity. When H₂O₂ is present, peroxidase can catalyze the oxidation of TMB. Reaction, when His-Au NCs is mixed with H₂O₂ and TMB, the solution rapidly changes from colorless to blue, and the maximum absorption wavelength of the mixture is 652 nm. This is due to the TMB is oxidized to oxTMB and the solution is blue. These results indicated that His-Au NCs are capable of oxidizing TMB to develop color and have peroxidase-like properties (Liu Y. et al., 2017). Lin et al. compared the catalytic activity of unmodified Au NCs and studied the effect of amino-modified gold clusters (NH₂-Au NCs) and citric acid-modified gold clusters (itrate-Au NCs) on the substrates ABTS and TMB. It was found that the surface unmodified Au NCs has the highest peroxidase activity. NH₂-Au NCs have higher catalytic activities for ABTS than citrate-Au NCs, while NH₂-Au NCs have lower catalytic activity for TMB than itrate-Au NCs. For the surface modified by Au NCs has different charges, it exhibits different variation, and the ability to adsorb the oxidized substrate is different, thus showing the difference in catalytic activity. The biocompatibility of Au

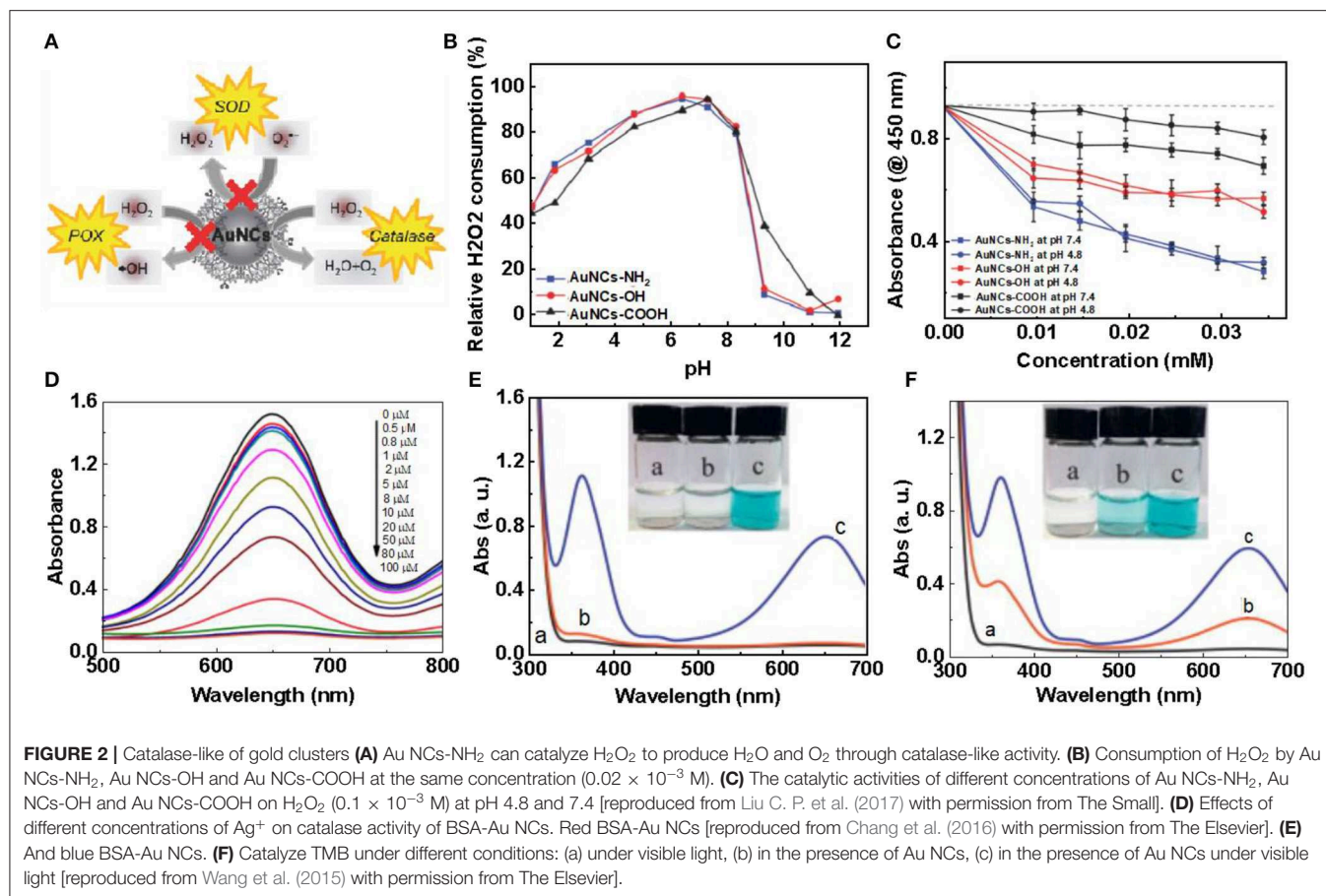


NCs was adjusted by selecting different ligands (Liu et al., 2016). Wang's team prepared bovine serum albumin (BSA) modified Au NCs. In order to improve the peroxidase-like activity of Au NCs (Wang et al., 2011). Wen et al. used the horseradish peroxidase properties of gold nanoclusters to detect H_2O_2 (Wen et al., 2011). Jiang et al. discovered and applied precious metal nanoclusters. They report that gold chains of ferritin iron (Au-Ft) can produce a blue reaction by catalyzing the oxidation of TMB by H_2O_2 . Compared to the native enzyme, Au-Ft exhibits higher activity and pH, temperature range, and the catalyzed reaction follows typical Mie kinetics. The lower K_m value ($0.097 \mu\text{M}$) was exhibited by the Au-Ft kinetic parameters, and the specific activity for TMB oxidation exceeded HRP. According to these findings, Au-Ft was used as a peroxide mimic enzyme to perform glucose spectrometry. Photometric analysis the system exhibited acceptable repeatability and high specificity (Liu J. et al., 2017).

Catalase-Like Property of Gold Clusters

Catalase is an important enzyme that prevents oxidative damage of cells by reactive oxygen species (Glorieux and Calderon, 2017). For Au NCs, Au^{2+} is first reduced by a H_2O_2 to form Au^+ , which is accompanied by the production of protons and O_2 . After that, another H_2O_2 can be combined with oxygen vacancies to oxidize Au^+ to Au^{2+} and release H_2O . This completes the simulation of hydrogen peroxide. Most nanomaterial-based peroxidase mimetics typically exhibit enzymatic activity under alkaline conditions rather than under physiological conditions (Góth et al., 2004; Glorieux and Calderon, 2017). Under the basic conditions of OH pre-adsorption, H_2O_2 first transfers one H^* to the pre-adsorbed OH to form HO_2^* and H_2O^* ; then, HO_2^* gives one H to the other H_2O_2 and finally produces H_2O^* and O_2^* (Figure 1C). Therefore, hydrogen peroxide-based activity can be observed under alkaline conditions (Li et al., 2015). He et al. (2013) demonstrated the intrinsic catalase activity of Au NCs using electron spin resonance spectroscopy combined

with spin trapping and spin labeling. Under normal and basic conditions, Au NCs exhibit inherent catalase catalytic activity because Au NCs can convert H_2O_2 to H_2O and O_2 . However, under acidic conditions, the catalase-like activity of Au NCs is significantly reduced, and once trapped in organelles, such as endosomes ($\text{pH} \approx 5.5$) and lysosomes ($\text{pH} \approx 4.8$), hydroxyl groups are produced. Free radicals ($\cdot\text{OH}$) can easily induce apoptosis. Therefore, the catalase-like activity of Au NCs is limited (He et al., 2013). To change this situation, Liu et al. studied amine-terminated macromolecularly encapsulated gold nanoclusters (Au NCs- NH_2). Au NCs- NH_2 exhibits good catalase activity at physiologically acidic pH values (Liu C. P. et al., 2017). Figure 2A is a schematic diagram showing the enzymatic activity of Au NCs- NH_2 , which can catalyze the production of O_2 by H_2O_2 by catalase activity. They changed the different groups of dendrimers, added Au NCs-OH and Au NCs-COOH, and studied the relative H_2O_2 consumption of Au NCs- NH_2 , Au NCs-OH and Au NCs-COOH under different solution pH values. A comparison of the amounts, they still have significant catalase-like activity at pH 4.8-7.4 (Figure 2B). By comparing the catalytic activities of Au NCs- NH_2 , Au NCs-OH and Au NCs-COOH at various concentrations of H_2O_2 at pH 4.8 and pH 7.4, the effect of Au NCs- NH_2 was significantly better (Figure 2C). Fan et al. synthesized a derivative protein (apoFt) as a nanoreactor to obtain Au-apoFt with adjustable size and uniform dispersion. The catalytic activity of both was observed at pH and temperature compared to natural catalase. As the pH and temperature increase, the enzyme activity of Au-apoFt increases. The increase in pH may be due to the presence of OH⁻ which promotes the dehydrogenation step of water in the catalytic reaction, showing an increase in enzyme activity. With rising of temperature, the increase in the enzyme activity of Au-apoFt may be due to an increase in the rate of molecular motion, which allows more H_2O_2 to adsorb to the surface of the Au nanoclusters, showing an increase in catalytic activity. Liu et al.

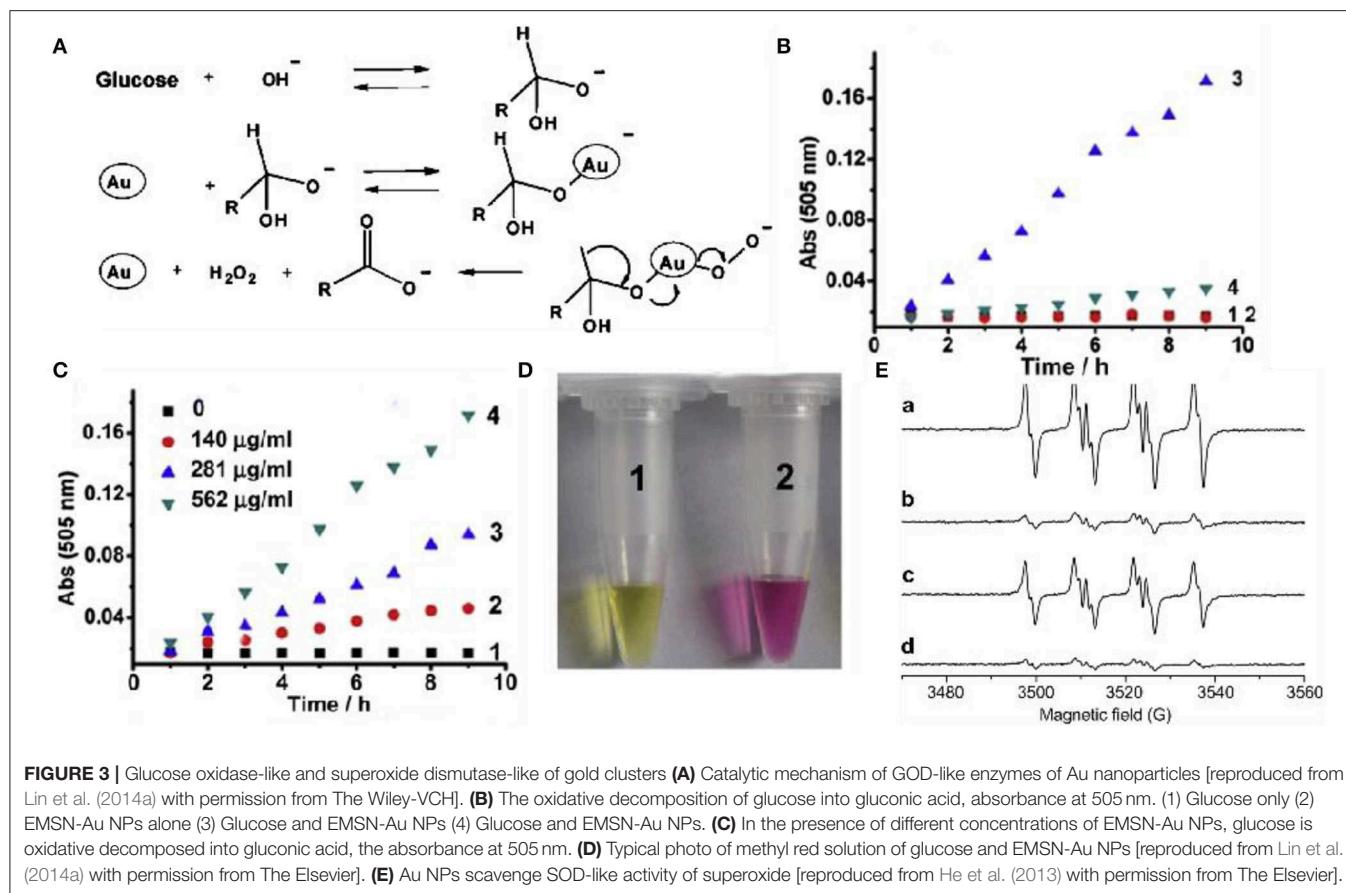


found that bovine serum albumin-protected gold nanoclusters (BSA-Au NCs) can sensitively measure H₂O₂ concentration (Figure 2D). Red BSA-Au NCs have no catalytic activity for TMB in the presence of oxygen but no light, and have catalytic activity in the presence of oxygen and light (Figure 2E); blue BSA-Au NCs have a slight catalytic activity for TMB in the presence of oxygen but no light, and have strong catalytic activity when oxygen and light coexist (Figure 2F). The experiment shows that light can stimulate the catalytic activity of Au NCs (Wang et al., 2015).

Glucose Oxidase-Like Property and Superoxide Dismutase-Like Property of Gold Clusters

Glucose oxidase (GOD) is widely distributed in animals and plants and microorganisms, and can specifically catalyze the production of glucose into gluconic acid and hydrogen peroxide under aerobic conditions (Gibson et al., 1964; Wilson and Turner, 1992; Bankar et al., 2009; Luo et al., 2010). At present, the application fields of GOD are constantly expanding, and the demand in domestic and foreign markets has increased dramatically. Low yield, low enzyme activity, and complex detection methods are the limiting factors for GOD industrialization. A lot of work has been done at home and

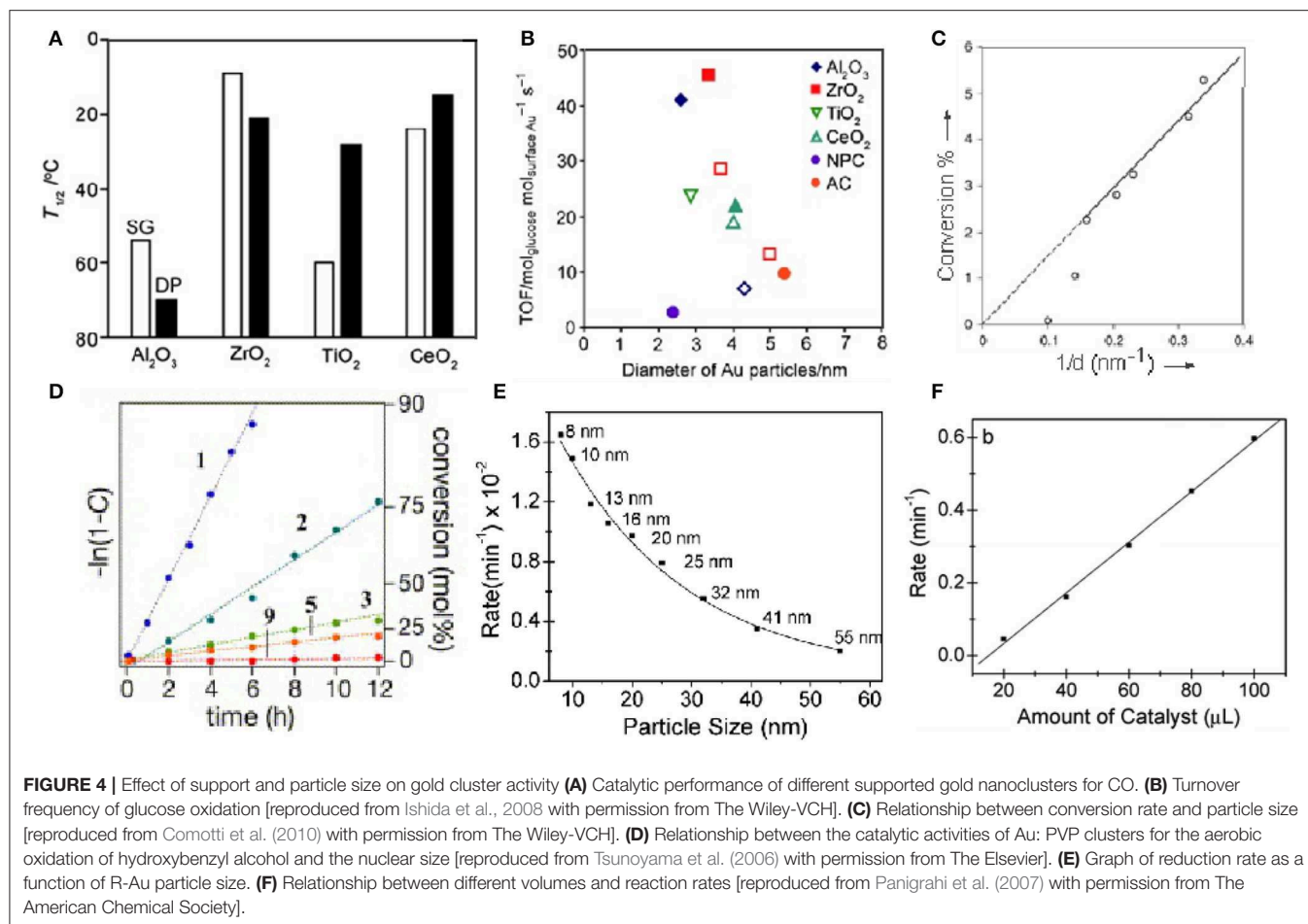
abroad and significant progress has been made. Recent studies have proved that gold clusters have excellent GOD-like enzyme activity. Luo et al. reported an interesting autocatalytic, self-limiting system that controls the controlled growth of Au NCs with GOD-like (Luo et al., 2010). In this system, Au NCs can serve as both seeds and catalyst, that is, the Au NCs catalyzed glucose oxidation *in situ* produced H₂O₂, and induced the Au NCs seeds in the presence of gold chloride ions. More importantly, the growth of Au NCs is internally regulated by two negative feedback factors, the reduced size-dependent activity of Au NCs and the glucose-induced surface passivation of the products, leading to rapid self-limiting systems. Pandey et al. (2007) used chemical synthesis to covalently combine GOD with the surface of gold nanoclusters to form a GOD-Au NCs complex, which improves the catalytic activity of GOD, improves stability, and enhances the enzyme response temperature and pH durability (Xia et al., 2013). However, this method only improves the performance of the enzyme and does not fundamentally solve the problem. The activity of the enzyme is still restricted by a series of factors, and other substances are introduced into the reaction system, which makes the entire system more complicated. Rossi et al. found that in the presence of O₂, glucose can be catalyzed by “bare” gold nanoclusters to produce gluconic acid and H₂O₂ (Pina et al., 2011). Based on the promotion of alkali and the production of H₂O₂, they proposed the mechanism



of molecular activated gold catalysis (Figure 3A). The surface of the gold atom interacts with the hydrated glucose anion to form electron-rich gold, which effectively activates molecular oxygen through nucleophilic attack. O_2 and gold intermediates $Au^{2+} + -O_2^{2-}$ or $Au + -O_2^-$ can act as a bridge for the conversion of electrons from glucose to hydrogen peroxide. Thereby the final reaction product is formed. Lin et al. studied mesoporous silica-encapsulated gold nanoclusters (EMSN-Au NPs) with GOD properties through UV-Vis (Figures 3B,C) (Lin et al., 2014a). First, the GOD mimicking activity of EMSN-Au NPs in solution was evaluated. Glucose is catalyzed by them in the presence of O_2 to produce gluconic acid. During the experiment, they used methyl red to detect the change in pH of the solution, as shown in Figure 3D. The results further confirmed that gluconic acid was indeed produced in the reaction catalyzed by EMSN-Au NPs. Superoxide dismutase (SOD) is a kind of antioxidant metal enzyme *in vivo*. It can catalyze superoxide anion free radical disproportionation to generate hydrogen peroxide and oxygen, which is very important in anti-oxidation. Gold nanoclusters decompose O_2^{2-} into molecular oxygen (O_2) and hydrogen peroxide (H_2O_2) through a cyclic redox electron transfer mechanism, thereby eliminating O_2^{2-} activity. Weiwei et al. verified the SOD activity of Au NPs through ESR experiments (Figure 3E) (He et al., 2013).

Effect of Support and Particle Size on Gold Cluster Activity

The enzymatic activities of nanomaterials are related to size (Mavrikakis et al., 2000; Lopez et al., 2004; Molina and Hammer, 2005; Miller et al., 2006; Zhou et al., 2010; Brodersen et al., 2011). It is possible to regulate the activity of nanomaterials by controlling their size, which has been confirmed in many studies. For gold nanomaterials as an example; the enzyme-like activity of gold nanoclusters is stronger than that of gold nanoparticles. Li et al. compared the enzyme-like activities of gold nanozymes with different structures and morphologies, including Au nanoclusters, Au nanoparticles, and Au nanotubes. At the same specific surface area, the Au nanoclusters had the strongest enzyme-like activity, while the Au nanotubes had the weakest enzyme-like activity. Through the study and analysis, they concluded that the differences in the enzyme-like activity of materials may be related to the differences in the crystal planes (Li et al., 2015). Corma et al. supported gold atoms on functionalized carbon nanotubes and explored their catalytic properties for phenol. They have catalytic activity equivalent to that of thiol oxidase. The catalytic activity also decreases with the increase of the size of the gold cluster, until it almost disappeared. According to theoretical calculations, smaller gold clusters can activate thiophenol and O_2 and are therefore active, while larger nanoparticles are inactivated by alkoxides and



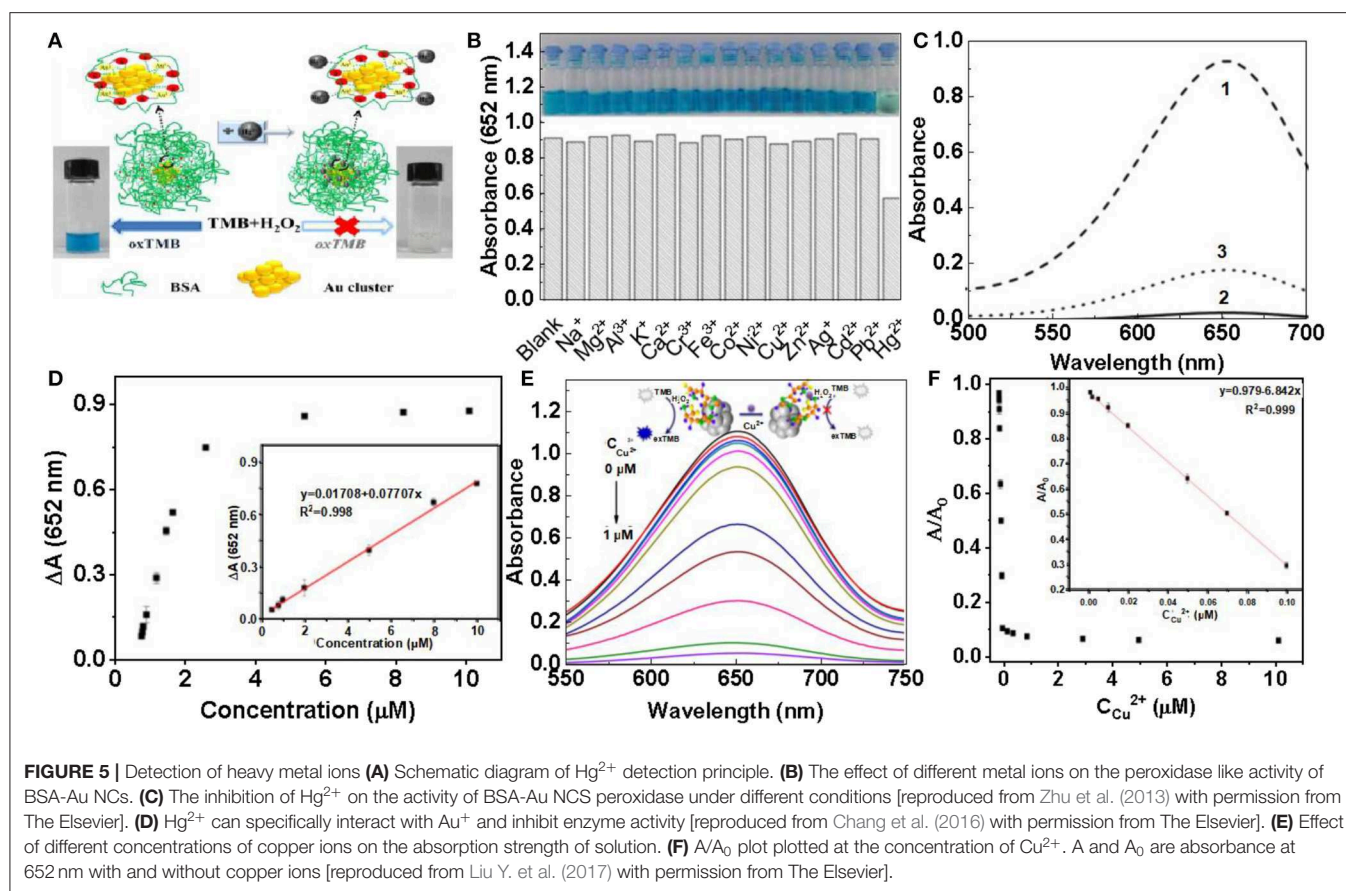
lose their activity (Corma et al., 2013). Tamao et al. tested the catalytic performance of gold nanoparticles on CO under different loads (**Figure 4A**). Compared with ZrO₂, TiO₂ and CeO₂, Al₂O₃ has a lower catalytic activity (Ishida et al., 2008). Oxygen vacancies can be formed at the peripheral interface of the Au particles. When gold nanoclusters are deposited on carbon materials and polymers, they lose their catalytic activity at temperatures above 120°C. In the CO oxidation reaction, the catalytic activity of glucose oxidation observed on Au catalysts is more affected by Au particle size than by carrier properties, and is related to the turnover frequency (TOF) of Au atoms on the surface (**Figure 4B**). Rossi et al. studied the effect of particle size on the catalytic activity by using gold clusters (3–10 nm) dispersed in water (Comotti et al., 2010). According to their research, the catalytic activity of glucose oxidase increases with decreasing particle size (**Figure 4C**). They first showed that gold nanoclusters (Au NCs) can catalyze the oxidation of glucose in the presence of O₂ to produce gluconic acid and H₂O₂. In contrast, other metal nanomaterials tested, such as Cu, Ag, Pd, and Pt, did not show significant oxidase-like activity under similar conditions (Comotti et al., 2010; Quan et al., 2015). Lin et al. compared the CAT activity of several clusters experimentally, and the results showed that the CAT activity of Pt and Pd was better than that of Au and Ag. Otherwise, the SOD-like and CAT-like activity of Au and Pt nanozymes

increased under alkaline conditions and decreased under acidic conditions (Lin et al., 2014b). Tsunoyama et al. prepared a group of monodisperse gold clusters (Au: PVP) through seed-mediated growth in the presence of polyvinylpyrrolidone (PVP) (Tsunoyama et al., 2006). The catalytic activity of Au: PVP clusters on hydroxybenzyl alcohol decreases with increasing core size (**Figure 4D**). Panigrahi et al. studied a core-shell Nano composite (R-Au) (**Figure 4E**) and derived the relationship between reduction rate and gold nanoclusters size (Panigrahi et al., 2007). The reaction rate decreases with increasing particle size. When the particle size is increased to 32 nm, as the particle size increases, the decline rate tends to be gentle. They believe that as the particle size increases, the decrease in catalytic performance is due to the increase in particle surface roughness. Therefore, the smaller the particle size, the higher the catalytic activity (**Figure 4F**).

APPLICATION OF GOLD CLUSTERS

Detection of Heavy Metal Ions

At present, heavy-metal contamination has caused great threats to the human health and our living environment. It is of great significance for the detection of heavy metals, with high selectivity of heavy metals (Bhan and Sarkar, 2005; Gallardo et al., 2014; Martin and Griswold, 2018). It is well-known



that mercury is a toxic heavy metal and widely found in the environment (Ercal et al., 2001). Mercury ion (Hg^{2+}) is the most common heavy metal ions. Even at very low concentrations, its destructive properties can affect the brain, god system and kidney (Bhan and Sarkar, 2005). Therefore, it is necessary to establish a fast, simple and sensitive method to detect Hg^{2+} in the environment. Zhu et al. found that Hg^{2+} has a selective inhibitory effect on the peroxidase activity of BSA-Au clusters (Zhu et al., 2013). The effect of common metal ions on the catalytic activity of BSA-Au was investigated using fluorescence quenching (Figure 5A). At the same concentration, Na^+ , Fe^{3+} , Co^{2+} , Ag^+ , Mg^{2+} , Al^{3+} , K^+ , Ca^{2+} , Cr^{3+} , Ni^{2+} , Cu^{2+} , Zn^{2+} , Cd^{2+} , Pb^{2+} to BSA-Au NCs have no effect on peroxidase-like activity. Hg^{2+} can inhibit the peroxidase-like activity of BSA-Au NCs and hardly catalyze the color reaction of TMB and H_2O_2 (Figure 5B). Similarly, the absorption spectrum of the reaction solution at 652 nm can also indicate that Hg^{2+} can inhibit the catalytic activity of BSA-Au NCs (Figure 5C, curve 2). Chelation of EDTA with Hg^{2+} can reduce the inhibitory effect of Hg^{2+} on the catalytic activity of BSA-Au NCs (Figure 5C, curve 3). Because mercury is easily complexed with sulfur, mercury ions (Hg^{2+}) can combine with cysteine through Hg-S bond to form Hg-Cys complex. The affinity of cysteine to Hg^{2+} was significantly higher than that of other metal ions. Based on this mechanism, Ding et al. constructed fluorescence quenching of citrate-modified Au NCs to detect Hg^{2+} in tap water (Ding et al., 2012). Then, Qi et al. reported a probe that can paper-based visualization of Hg^{2+} based on conjugates of Tb^{3+} /BSA-Au

NCs. The probe can be highly complexed with Hg^{2+} via Hg-S bonds, and therefore has excellent selectivity. The method is simple and easy to operate, with only a ultraviolet lamp needed, which can be greatly promoted in practical applications (Qi et al., 2015). Using similar sensing mechanism, Lin et al. constructed a method to detect Hg^{2+} and methylmercury in seawater based on Lys protected Au NCs probe, and Xu et al. also used lysozyme-modified Au NCs to visually detect Hg^{2+} in water by visual and fluorescent colorimetry (Xu et al., 2015). The Zhu working group provided us with a colorimetric method that can detect Hg with high sensitivity and selectivity. They also detected of different working fluids and the influence of common metal ions on the catalytic activity. Finally, Hg^{2+} can specifically interact with Au^+ to inhibit enzyme activity (Figure 5D) (Zhu et al., 2013). Liu et al. combined the peroxidase-like nanozymes activity of gold nanoclusters with the double-stranded nature of amino acids, and proposed a simple, sensitive and selective method for the detection of Cu^{2+} and histidine (His). The addition of different concentrations of Cu^{2+} can inhibit the peroxidation of histidine-gold nanoclusters (His-Au NCs) to varying degrees (Figure 5E). The absorbance of the solution at 652 nm gradually decreases with increasing Cu^{2+} concentration, so this method can be used to detect the concentration of Cu^{2+} in the solution. According to this calibration curve, Cu^{2+} can be measured with high sensitivity (Figure 5F) (Liu Y. et al., 2017). Although gold nanocluster probes with enzyme activity have been widely developed in recent years, many problems still need to be solved. First, most of the probes with enzyme activity controlled by

metal ions are POD activity, while few probes are available for other types of enzyme activities. In addition, the sensor mechanism of the probes is relatively single, and more types of mechanisms need to be developed to design the probes. Finally, there is little research on the application in biological matrix or *in vivo*, which is very important for the biological application of enzyme activity.

Anion Detection

Inorganic anions are widely present in ecosystems, but it should be noted that most inorganic anions have two-sided effects on the ecological environment and human health (Velizarov et al., 2004). Shojaeifard et al. tested environmental water and CN in human serum based on a combination of Au NCs and copper (II)-phthalocyanine complexes. Under the condition of CN^- , the binding of Au NCs to the copper (II)-phthalocyanine complex is destroyed, so the fluorescence of the copper (II)-phthalocyanine complex quenched by Au NCs is restored, thus forming a stable $[\text{Au}(\text{CN})_2]^-$. The method has good selectivity, high sensitivity and is suitable for popularization (Shojaeifard et al., 2016). Liu et al. also constructed a method based on Au NCs to detect CN^- in environmental water samples, and successfully used to detect CN^- in food sand samples and biological samples (Liu et al., 2010). Xiong et al. synthesized BSA-Au NCs and successfully detected Cl^- in tap water with a detection limit of $0.50 \mu\text{mol/L}$ (Xiong et al., 2015). Wang et al. used glutathione-coated Au NCs as probes to construct a method for detecting I^- in water with a detection limit of 400 nmol/L . This method can selectively identify I^- from 12 common anions such as F^- , Cl^- , Br^- (Yang et al., 2014). At the same time, Chang group used DNA as template to synthesize gold/silver nanozymes. Clusters were used to detect S^{2-} in hot spring and seawater samples. This method specifically recognized S^{2-} from Au^{3+} , Ag^+ and DNA in the presence of NaBH_4 . The quenching mechanism is that S can interact with gold and silver atoms, thus resulting in changes in the conformation of the template DNA (Chen et al., 2011). At present, gold clusters have been used to detect anions *in vitro* more thoroughly and sensitively, but few studies have been done in combination with organisms. The biological environment is more complex, so anion detection *in vivo* will be a challenge. In addition, the role of gold clusters in organisms should not only be a function of detection, but also require us to develop more properties of gold clusters for the diagnosis and treatment of organisms.

Biological Application of Gold Clusters

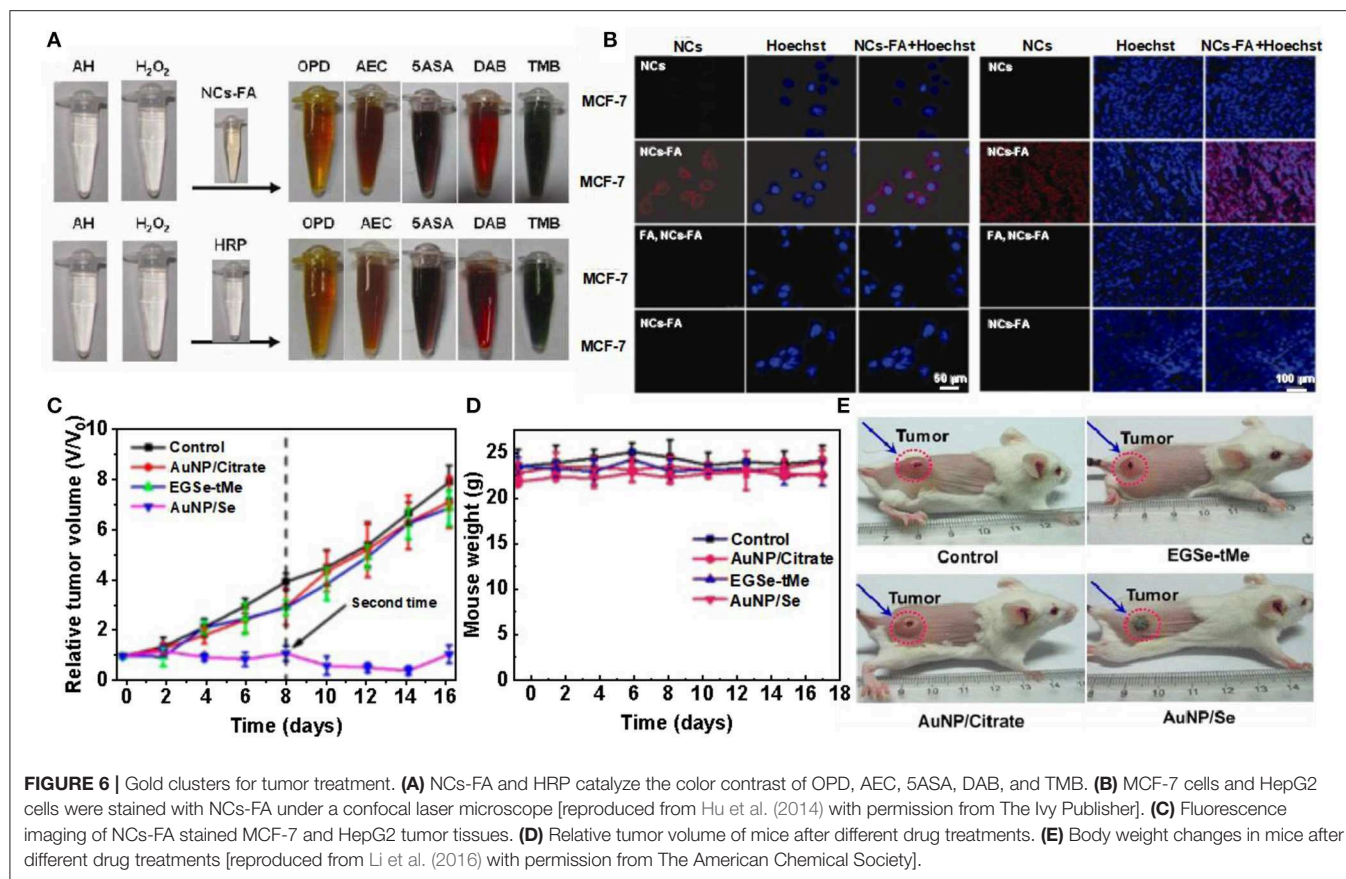
Tumor Treatment

Nanoprobe with enzymatic properties have been attracting increasing attention in early screening and diagnosis of cancer (Schaller and Graf, 2004; Spichiger-Keller, 2008; He et al., 2010; Chi et al., 2012; Li and Xiaogang, 2015). In order to achieve the specificity and high accuracy of tumor detection, it was necessary to design and prepare an enzyme-simulated nanoprobe with tumor targeting, high enzyme activity and containing luminescent properties. Hu et al. described the folate receptor based gold nanoclusters (NCs-FA), which are novel luciferase mimic nanoprobe with high stability, low cytotoxicity and

high enzyme activity. O-Phenylenediamine (OPD), 3-amino-9-ethylcarbazole (AEC), 5-aminosalicylic acid (5ASA) and 3,3',5,5'-tetramethylbenzidine (TMB) can be used NCs-FA catalyzes to produce red, brown, brown, and blue. Experiments prove that NCs-FA nanoprobe has peroxidase activity (Figure 6A). To visualize the uptake of NCS-FA nanoprobe by cells, MCF-7 and HepG2 cells were stained with NCs-FA nanoprobe (NC) and observed under a focused laser microscope (Figure 6B) (Hu et al., 2014). The probes (NC) stained MCF-7 and HepG2 tumor tissues and observed them by fluorescence microscopy (Figure 6C). It confirmed that NCs-FA nanoprobe target tumor cells *via* FR. Au NPs have low anticancer activity and are widely used in drug carriers, biological imaging, and other fields. The new selenium-containing molecule (EGSe-TME) has low anticancer activity, but the combination of NCs-FA and EGSe-TME has produced a system with good anticancer activity. Li et al. synthesized Au NP/Se. In order to explore its cytotoxicity, tumor mice were injected with PBS, Au NP/citrate, EGSe-tMe, and Au NP/Se on day 0 and 8, respectively. Compared with the tumor volume of the control group, mice treated with Au NP/citrate, EGSe-tMe had less obvious tumor growth inhibition effect, and however, mice treated with Au NP/Se showed tumor growth strong inhibitory effect (Figure 6D). There was no significant change of mice treated with Au NP/Se (Figure 6E). And photographs of mice after administration of different drugs on day 10 were observed, which showed that the systemic toxicity of Au NP/Se was low (Li et al., 2016). However, there are still some problems in the development of nanoprobe for tumor microenvironment. Further binding to ligands may help to reduce toxicity and guide targeting, but can affect the catalytic activity and subsequent metabolism of the nanozymes, as well as the microenvironment of the organisms. Although nanoprobe have achieved good results in animal experiments, the differences between animals and humans are huge, and their clinical applications need to be further developed and utilized.

Brain Therapy

The toxicity of nanozymes has caused widespread concern (Zhang et al., 2018). Liu et al. found that amino-terminated gold nanoclusters (Au NCs-NH₂) have low cytotoxicity and can protect primary neurons from oxidative damage (Liu et al., 2016). The peroxidase-like activity of Au NCs-NH₂ is inhibited by the polymerized 3-amines, and thus exhibits catalase-like activity, which decomposes H₂O₂, thereby providing neurons with protection against oxidative damage (Figure 7A) (Mukherjee et al., 2010). Compared to the control, H₂O₂ excitation resulted in significant cell death and less red fluorescence. Neuronal cells were pre-treated with Au NCs-NH₂ and showed red fluorescence similar to that of the control group after challenge with H₂O₂. The same is true for neurons treated with Au NCs-NH₂ alone, further demonstrating the low cytotoxicity of Au NCs-NH₂ (Figure 7B) (Wang et al., 2013). Cell viability in different treatment groups was determined by MTT assay and treatment with H₂O₂ alone (100×10^{-6} or 200×10^{-6} M) resulted in a significant decrease in neuronal cell viability. Pretreatment with Au NCs-NH₂ followed by challenge with H₂O₂, the cells remained viable and the results were similar to the untreated



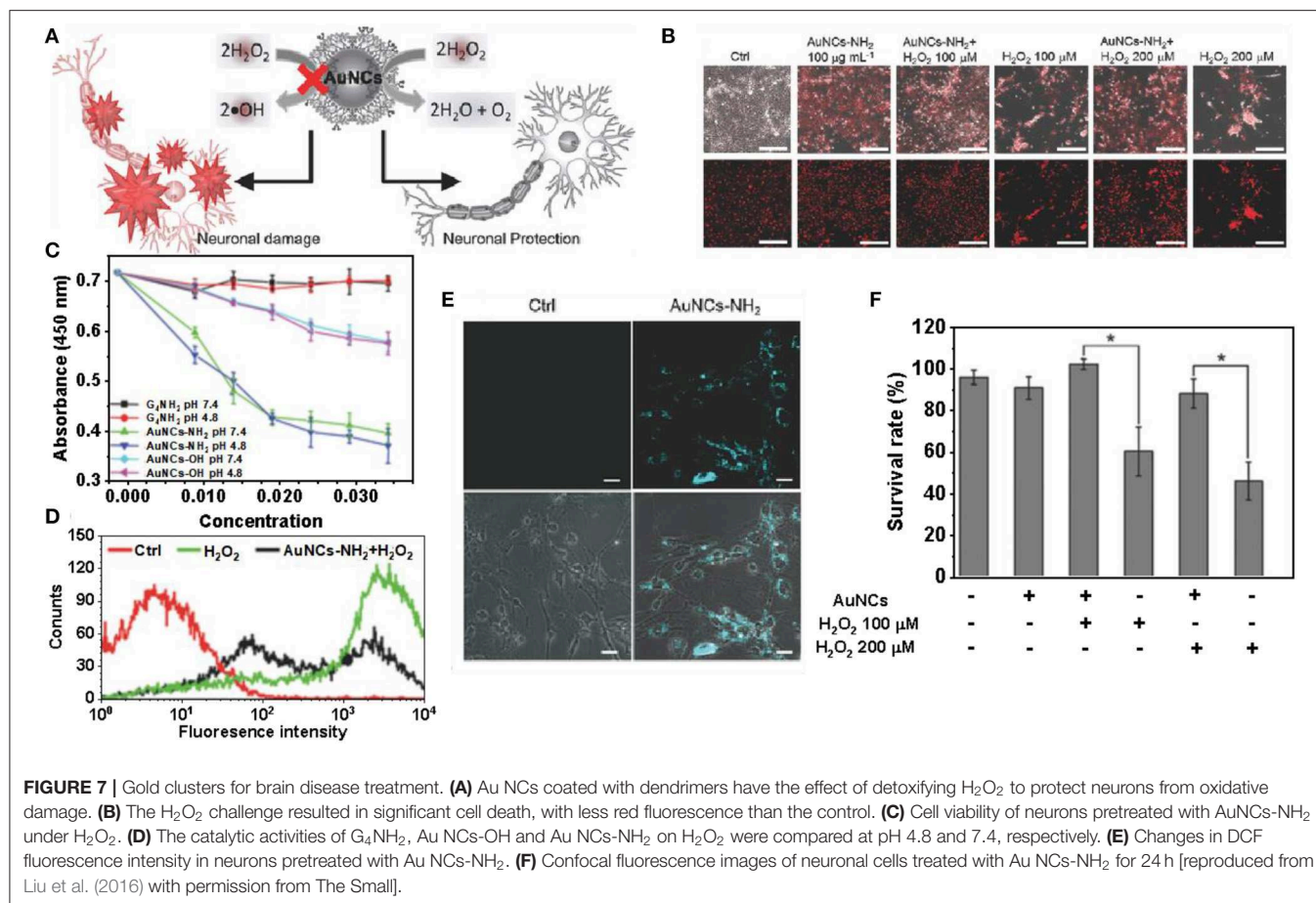
controls. This indicates that pretreatment of primary neuronal cells with Au NCs-NH₂ have resistance to H₂O₂ induced toxicity (Figure 7C) (Huang et al., 2004). The catalase-like activities of Au NCs-NH₂ and Au NCs-OH prepared from different end groups of dendrimers were not affected at different pH, but the catalytic performance of Au NCs-NH₂ for H₂O₂ was significantly better than that of Au NCs-OH (Figure 7D) (Jao et al., 2010). The level of H₂O₂ in cells after H₂DCFDA staining was quantified by flow cytometry, and the protective effect of NCs-NH₂ in H₂O₂ treated neurons was obtained (Figures 7E,F). The fluorescence intensity of neurons pretreated with Au NCs-NH₂ was significantly increased compared to the control (Graf et al., 2004). Although the catalytic activity of nanozymes is closely related to their surface properties, the mechanism of enzymelike activity of its surface coating is poorly understood. Once the nano-enzyme loses its surface accessibility, its catalytic performance will be inhibited, thus affecting its application in brain therapy and other aspects. Currently, biomedical research on gold nanoclusters without surface coating is limited due to concerns about their toxicity and stability.

CONCLUSION

In this article, we attempt to give a comprehensive overview based on gold nanoclusters. We have summarized the characteristics of

enzymes such as glucose oxidase, peroxidase, catalase, superoxide dismutase, etc. The applications of gold nanoclusters in ion detection, tumor treatment and brain treatment were analyzed. This enzymatic property of gold clusters is derived from the functional groups present on the gold itself or on the surrounding monolayer. Although the development of gold clusters catalysts has made encouraging progress, the overall performance of these artificial catalytic systems is often not comparable to natural catalysts. To this end, the following aspects are awaiting implementation:

- (1) In recent years, with the development of nanotechnology, we can control the size and shape of nanoparticles by including hydrogen reduction, self-assembly, porous support matrix and surfactant assisted methods to adjust their catalytic activity. Because of the size dependence of gold nanomaterials, this allows us to conduct directional manipulation of surface properties. Because the surface charge and other parameters have great influence on the cell adhesion behavior, the preparation of gold nanoclusters enzyme with atomic precision is a promising method to affect the biological activity. In addition, catalysts with atomic precision are more conducive to revealing the relationship between electronic structure and properties. At the same time, the sources of catalytic activity of functional groups, and the future work may continue to focus on



changes in functional groups present at catalytic sites, resulting in highly active catalysts. More importantly, we hope to open up new strategies to significantly improve catalytic performance and biosafety through the regulation of three changes in atomic accuracy, catalytic sites and functional groups.

- (2) Although rapid progress has been made in the development of nanomaterials with enzyme mimicking activity, it is unclear how widely these materials are used *in vivo* and clinically. So far, it is uncertain whether gold nanoclusters with enzyme-like activity can significantly replace many naturally occurring enzymes. All endogenous enzymes in our bodies are as a whole system and work interdependently. Any artificial substitutes, including nanoclusters, may not be suitable for the system and may cause serious side effects. During the expression of enzyme-like functions, nanoclusters can generate free radicals, causing toxicological effects. Therefore, it is worthy of further study to make full use of the beneficial effect of the enzyme-like activity of the nanoclusters and explore their application *in vivo*.
- (3) Although many researchers have demonstrated the therapeutic effects of nanozymes systems through cell and animal experiments, it is still difficult to explain the mechanism of nanozymes systems *in vivo* through these biological experiments. For example, quantifying reaction

kinetics *in vivo* is not an easy task, although the available evidence supports the ultimate therapeutic outcome of these nanozymes. We can only know the results, but not the processes in the body. In recent years, multifunctional optical probes have been widely developed to detect the concentration and distribution of specific biochemical substances in the biological environment. Therefore, we may need to develop multifunctional nanometer enzyme probes, such as using the luminescence of gold clusters for imaging or sensing (Such as detecting markers of neurological disease), and then combining its enzyme-like properties for treatment, which can achieve the purpose of combining diagnosis with treatment, providing powerful help for the study of the distribution and mechanism of action of nanomaterials in living organisms. These diagnostic reagents are expected to help accurately characterize the catalytic process *in vivo*, and ultimately comprehensively clarify the relationship between the composition, structure, and *in vivo* properties of nanozymes drugs.

AUTHOR CONTRIBUTIONS

SL wrote the first draft. HL and X-DZ modified the manuscript content and format. YZ and WL modified the syntax.

FUNDING

This work was financially supported by the National Natural Science Foundation of China (Grant No. 91859101, 81971744,

U1932107, 81673106, and 81471786), CAMS Innovation Fund for Medical Science (2017-12M-1-012) and National Natural Science Foundation of Tianjin (No. 19JCZDJC34000), the Innovation Foundation of Tianjin University.

REFERENCES

- Arnold, F. H., Wintrode, P. L., Miyazaki, K., and Gershenson, A. (2001). How enzymes adapt: lessons from directed evolution. *Trends Biochem. Sci.* 26, 100–106. doi: 10.1016/S0968-0004(00)01755-2
- Bankar, S. B., Bule, M. V., Singhal, R. S., and Ananthanarayan, L. (2009). Glucose oxidase—an overview. *Biotechnol. Adv.* 27, 489–501. doi: 10.1016/j.biotechadv.2009.04.003
- Barzegar, A., and Moosavi-Movahedi, A. A. (2011). Intracellular ROS protection efficiency and free radical-scavenging activity of curcumin. *PLoS ONE* 6:26012. doi: 10.1371/journal.pone.0026012
- Bhan, A., and Sarkar, N. (2005). Mercury in the environment: effect on health and reproduction. *Rev. Environ. Health.* 20, 39–56. doi: 10.1515/REVEH.2005.20.1.39
- Brodersen, S. H., Grønberg, U., Hvolbæk, B., and Schiøtz, J. (2011). Understanding the catalytic activity of gold nanoparticles through multi-scale simulations. *J. Catal.* 284, 34–41. doi: 10.1016/j.jcat.2011.08.016
- Chang, H. T., Chen, P. C., Periasamy, A. P., Harroun, S. G., and Wu, W. P. (2016). Photoluminescence sensing systems based on copper, gold and silver nanomaterials. *Coordin. Chem. Rev.* 320, 129–138. doi: 10.1016/j.ccr.2015.12.002
- Chang, T. M. S. (2013). *Biomedical Applications of Immobilized Enzymes and Proteins*. Quebec City, QC: Springer.
- Chen, J., Huang, L., Wang, Q., Wu, W., Zhang, H., Fang, Y., et al. (2019). Bio-inspired nanzyme: a hydratase mimic in a zeolitic imidazolate framework. *Nanoscale*. 11, 5960–5966. doi: 10.1039/C9NR01093A
- Chen, W.-Y., Lan, G.-Y., and Chang, H.-T. (2011). Use of fluorescent DNA-templated gold/silver nanoclusters for the detection of sulfide ions. *Anal. Chem.* 83, 9450–9455. doi: 10.1021/ac202162u
- Chen, Z., Xu, L., Liang, Y., and Zhao, M. (2010). pH-sensitive water-soluble nanospherical imprinted hydrogels prepared as horseradish peroxidase mimetic enzymes. *Adv. Mater.* 22, 1488–1492. doi: 10.1002/adma.200903122
- Chi, X., Huang, D., Zhao, Z., Zhou, Z., Yin, Z., and Gao, J. (2012). Nanoprobes for *in vitro* diagnostics of cancer and infectious diseases. *Biomaterials* 33, 189–206. doi: 10.1016/j.biomaterials.2011.09.032
- Comotti, M., Pina, C. D., Matarrese, R., and Rossi, M. (2010). The catalytic activity of “naked” gold particles. *Angew. Chem. Int. Edit.* 116, 5936–5939. doi: 10.1002/ange.200460446
- Corma, A., Concepción, P., Boronat, M., Sabater, M. J., Navas, J., and Yacaman, M. J. (2013). Exceptional oxidation activity with size-controlled supported gold clusters of low atomivity. *Nat. Chem.* 5, 775–81. doi: 10.1038/nchem.1721
- Del Valle, E. M. (2004). Cyclodextrins and their uses: a review. *Process Biochem.* 39, 1033–1046. doi: 10.1016/S0032-9592(03)00258-9
- Ding, N., Zhao, H., Peng, W., He, Y., Zhou, Y., Yuan, L., et al. (2012). A simple colorimetric sensor based on anti-aggregation of gold nanoparticles for Hg²⁺ detection. *Colloid. Surface. A.* 395, 161–167. doi: 10.1016/j.colsurfa.2011.12.024
- Ding, Y., Zhao, J., Li, B., Zhao, X., Wang, C., Guo, M., et al. (2018). The CoOOH-TMB oxidative system for use in colorimetric and test strip based determination of ascorbic acid. *Microchim. Acta.* 185:131. doi: 10.1007/s00604-018-2675-z
- Ercal, N., Gurer-Orhan, H., and Aykin-Burns, N. (2001). Toxic metals and oxidative stress part I: mechanisms involved in metal-induced oxidative damage. *Curr. Top. Med. Chem.* 1, 529–539. doi: 10.2174/1568026013394831
- Erel, O. (2004). A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. *Clin. Biochem.* 37, 277–285. doi: 10.1016/j.clinbiochem.2003.11.015
- Fan, K., Xi, J., Fan, L., Wang, P., Zhu, C., Tang, Y., et al. (2018). *In vivo* guiding nitrogen-doped carbon nanzyme for tumor catalytic therapy. *Nat. Commun.* 9:1440. doi: 10.1038/s41467-018-03903-8
- Feng, J., Huang, P., Shi, S., Deng, K.-Y., and Wu, F.-Y. (2017). Colorimetric detection of glutathione in cells based on peroxidase-like activity of gold nanoclusters: a promising powerful tool for identifying cancer cells. *Anal. Chim. Acta.* 967, 64–69. doi: 10.1016/j.aca.2017.02.025
- Gallardo, C., Monrás, J., Plaza, D., Collao, B., Saona, L., Durán-Toro, V., et al. (2014). Low-temperature biosynthesis of fluorescent semiconductor nanoparticles (CdS) by oxidative stress resistant Antarctic bacteria. *J. Biotechnol.* 187, 108–115. doi: 10.1016/j.jbiotec.2014.07.017
- Gao, L., Zhuang, J., Nie, L., Zhang, J., Zhang, Y., Gu, N., et al. (2007). Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat. Nanotechnol.* 2, 577–83. doi: 10.1038/nnano.2007.260
- Geim, A. K., and Grigorieva, I. V. (2013). Van der Waals heterostructures. *Nature* 499, 419–425. doi: 10.1038/nature12385
- Gibson, Q. H., Swoboda, B. E., and Massey, V. (1964). Kinetics and mechanism of action of glucose oxidase. *J. Biol. Chem.* 239, 3927–3934.
- Gleiter, R., and Hopf, H. (2006). *Modern Cyclophane Chemistry*. Heidelberg; Braunschweig: John Wiley Sons.
- Glorieux, C., and Calderon, P. B. (2017). Catalase, a remarkable enzyme: targeting the oldest antioxidant enzyme to find a new cancer treatment approach. *Biol. Chem.* 398, 1095–1108. doi: 10.1515/hsz-2017-0131
- Góth, L., Rass, P., and Páy, A. (2004). Catalase enzyme mutations and their association with diseases. *Mol. Diagn.* 8, 141–149. doi: 10.1007/BF03260057
- Graf, E. R., Zhang, X. Z., Jin, S. X., Linhoff, M. W., and Craig, A. M. (2004). Neurexins induce differentiation of GABA and glutamate postsynaptic specializations via neuroligins. *Cell*, 119, 1013–1026. doi: 10.1016/j.cell.2004.11.035
- Griffith, L. G., and Naughton, G. (2002). Tissue engineering—current challenges and expanding opportunities. *Science* 295, 1009–1014. doi: 10.1126/science.1069210
- He, W., Wamer, W. G., Hu, X., and Wu, X. (2013). Intrinsic catalytic activity of Au nanoparticles with respect to hydrogen peroxide decomposition and superoxide scavenging. *Biomaterials* 34, 765–773. doi: 10.1016/j.biomaterials.2012.10.010
- He, W., Zhou, Y.-T., Wamer, W. G., Boudreau, M. D., and Yin, J.-J. (2012). Mechanisms of the pH dependent generation of hydroxyl radicals and oxygen induced by Ag nanoparticles. *Biomaterials* 33, 7547–7555. doi: 10.1016/j.biomaterials.2012.06.076
- He, X., Gao, J., Gambhir, S. S., and Cheng, Z. (2010). Near-infrared fluorescent nanoprobes for cancer molecular imaging: status and challenges. *Trends Mol. Med.* 16, 574–583. doi: 10.1016/j.molmed.2010.08.006
- Hu, D., Sheng, Z., Fang, S., Wang, Y., Gao, D., Zhang, P., et al. (2014). Folate receptor-targeting gold nanoclusters as fluorescence enzyme mimetic nanoprobes for tumor molecular colocalization diagnosis. *Theranostics* 4, 142–153. doi: 10.7150/thno.7266
- Huang, H., Starodub, O., McIntosh, A., Atshaves, B. P., Woldegiorgis, G., Kier, A. B., et al. (2004). Liver fatty acid-binding protein colocalizes with peroxisome proliferator activated receptor α and enhances ligand distribution to nuclei of living cells. *Biochemistry* 43, 2484–2500. doi: 10.1021/bi0352318
- Huang, L., Chen, J., Gan, L., Wang, J., and Dong, S. (2019a). Single-atom nanozymes. *Sci. Adv.* 5:ea5490. doi: 10.1126/sciadv.aav5490
- Huang, Y., Ren, J., and Qu, X. (2019b). Nanozymes: classification, catalytic mechanisms, activity regulation, and applications. *Chem. Rev.* 119, 4357–4412. doi: 10.1021/acs.chemrev.8b00672
- Ishida, T., Kinoshita, N., Okatsu, H., Akita, T., Takei, T., and Haruta, M. (2008). Influence of the support and the size of gold clusters on catalytic activity for glucose oxidation. *Angew. Chem.* 47, 9265–9268. doi: 10.1002/anie.200802845
- Jao, Y.-C., Chen, M.-K., and Lin, S.-Y. (2010). Enhanced quantum yield of dendrimer-entrapped gold nanodots by a specific ion-pair association and microwave irradiation for bioimaging. *Chem. Commun.* 46, 2626–2628. doi: 10.1039/b926364k

- Jiang, D., Ni, D., Rosenkrans, Z. T., Huang, P., Yan, X., and Cai, W. (2019). Nanozyme: new horizons for responsive biomedical applications. *Chem. Soc. Rev.* 48, 3683–3704. doi: 10.1039/C8CS00718G
- Jin, R. (2010). Quantum sized, thiolate-protected gold nanoclusters. *Nanoscale* 2, 343–362. doi: 10.1039/B9NR00160C
- Kim, Y., Johnson, R. C., and Hupp, J. T. (2001). Gold nanoparticle-based sensing of “spectroscopically silent” heavy metal ions. *Nano Lett.* 1, 165–167. doi: 10.1021/nl0100116
- Lequin, R. M. (2005). Enzyme immunoassay (EIA)/enzyme-linked immunosorbent assay (ELISA). *Clin. Chem.* 51, 2415–2418. doi: 10.1373/clinchem.2005.051532
- Li, J., Liu, W., Wu, X., and Gao, X. (2015). Mechanism of pH-switchable peroxidase and catalase-like activities of gold, silver, platinum and palladium. *Biomaterials* 48, 37–44. doi: 10.1016/j.biomaterials.2015.01.012
- Li, T., Li, F., Xiang, W., Yi, Y., Chen, Y., Cheng, L., et al. (2016). Selenium-containing amphiphiles reduced and stabilized gold nanoparticles: kill cancer cells via reactive oxygen species. *ACS Appl. Mater. Inter.* 8, 22106–22112. doi: 10.1021/acsami.6b08282
- Li, W., and Xiaogang, Q. (2015). Cancer biomarker detection: recent achievements and challenges. *Chem. Soc. Rev.* 44, 2963–2997. doi: 10.1039/C4CS00370E
- Liang, M., and Yan, X. (2019). Nanozymes: from new concepts, mechanisms, and standards to applications. *Acc. Chem. Res.* 52, 2190–2200. doi: 10.1021/acs.accounts.9b00140
- Liao, H., Liu, G., Liu, Y., Li, R., Fu, W., and Hu, L. (2017). Aggregation-induced accelerating peroxidase-like activity of gold nanoclusters and their applications for colorimetric Pb²⁺ detection. *Chem. Commun.* 53, 10160–10163. doi: 10.1039/C7CC05409B
- Lin, Y., Ren, J., and Qu, X. (2014a). Nano-gold as artificial enzymes: hidden talents. *Adv. Mater.* 26, 4200–4217. doi: 10.1002/adma.201400238
- Lin, Y., Ren, J., and Qu, X. (2014b). Catalytically active nanomaterials: a promising candidate for artificial enzymes. *Accounts Chem. Res.* 47, 1097–1105. doi: 10.1021/ar400250z
- Liu, C. P., Wu, T. H., Lin, Y. L., Liu, C. Y., Wang, S., and Lin, S. Y. (2016). Tailoring enzyme-like activities of gold nanoclusters by polymeric tertiary amines for protecting neurons against oxidative stress. *Small* 12, 4127–4135. doi: 10.1002/smll.201503919
- Liu, C. P., Wu, T. H., Liu, C. Y., Chen, K. C., Chen, Y. X., Chen, G. S., et al. (2017). Self-supplying O₂ through the catalase-like activity of gold nanoclusters for photodynamic therapy against hypoxic cancer cells. *Small* 13:1700278. doi: 10.1002/smll.201700278
- Liu, H., Hong, G., Luo, Z., Chen, J., Chang, J., Gong, M., et al. (2019). Atomic-precision gold clusters for NIR-II imaging. *Adv. Mater.* 31:1901015. doi: 10.1002/adma.201901015
- Liu, J., Wang, Z., Yan, X., and Jian, P. (2017). Metallic cobalt nanoparticles imbedded into ordered mesoporous carbon: a non-precious metal catalyst with excellent hydrogenation performance. *J. Colloid Interf. Sci.* 505, 789–795. doi: 10.1016/j.jcis.2017.06.081
- Liu, Q., Yang, Y., Lv, X., Ding, Y., Zhang, Y., Jing, J., et al. (2017). One-step synthesis of uniform nanoparticles of porphyrin functionalized ceria with promising peroxidase mimetics for H₂O₂ and glucose colorimetric detection. *Sensor. Actuat B-Chem.* 240, 726–734. doi: 10.1016/j.snb.2016.09.049
- Liu, S., Lu, F., Xing, R., and Zhu, J. J. (2011). Structural effects of Fe₃O₄ nanocrystals on peroxidase-like activity. *Chem. Eur. J.* 17, 620–625. doi: 10.1002/chem.201001789
- Liu, Y., Ai, K., Cheng, X., Huo, L., and Lu, L. (2010). Gold-nanocluster-based fluorescent sensors for highly sensitive and selective detection of cyanide in water. *Adv. Funct. Mater.* 20, 951–956. doi: 10.1002/adfm.200902062
- Liu, Y., Ding, D., Zhen, Y., and Guo, R. (2017). Amino acid-mediated ‘turn-off/turn-on’ nanozyme activity of gold nanoclusters for sensitive and selective detection of copper ions and histidine. *Biosens. Bioelectron.* 92, 140–146. doi: 10.1016/j.bios.2017.01.036
- Lopez, N., Janssens, T., Clausen, B., Xu, Y., Mavrikakis, M., Bligaard, T., et al. (2004). On the origin of the catalytic activity of gold nanoparticles for low-temperature CO oxidation. *J. Catal.* 223, 232–235. doi: 10.1016/j.jcat.2004.01.001
- Luo, W., Zhu, C., Su, S., Li, D., He, Y., Huang, Q., et al. (2010). Self-catalyzed, self-limiting growth of glucose oxidase-mimicking gold nanoparticles. *ACS Nano* 4, 7451–7458. doi: 10.1021/nn102592h
- Martin, S., and Griswold, W. (2018). Human health effects of heavy metals. *Internation. J.* 15, 1–6. doi: 10.14445/23939133/IJAC-V5I1P102
- Mavrikakis, M., Stoltze, P., and Nørskov, J. K. (2000). Making gold less noble. *Catal. Lett.* 64, 101–106. doi: 10.1023/A:1019028229377
- Meeuwissen, J., and Reek, J. N. (2010). Supramolecular catalysis beyond enzyme mimics. *Nat. Chem.* 2, 615–21. doi: 10.1038/nchem.744
- Miller, J., Kropf, A., Zha, Y., Regalbuto, J., Delannoy, L., Louis, C., et al. (2006). The effect of gold particle size on AuAu bond length and reactivity toward oxygen in supported catalysts. *J. Catal.* 240, 222–234. doi: 10.1016/j.jcat.2006.04.004
- Molina, L., and Hammer, B. (2005). Some recent theoretical advances in the understanding of the catalytic activity of Au. *Appl. Catal. A-Gen.* 291, 21–31. doi: 10.1016/j.apcata.2005.01.050
- Mu, X., He, H., Wang, J., Long, W., Li, Q., Liu, H., et al. (2019a). Carbogenic nanozyme with ultrahigh reactive nitrogen species selectivity for traumatic brain injury. *Nano Lett.* 19, 4527–4534. doi: 10.1021/acs.nanolett.9b01333
- Mu, X., Wang, J., Li, Y., Xu, F., and Long, W. (2019b). Redox trimetallic nanozyme with neutral environment preference for brain injury. *ACS Nano* 13, 1870–1884. doi: 10.1021/acsnano.8b08045
- Mukherjee, S. P., Davoren, M., and Byrne, H. J. (2010). *In vitro* mammalian cytotoxicological study of PAMAM dendrimers—towards quantitative structure activity relationships. *Toxicol. in vitro* 24, 169–177. doi: 10.1016/j.tiv.2009.09.014
- Pandey, P., Singh, S. P., Arya, S. K., Gupta, V., Datta, M., Singh, S. et al. (2007). Application of thiolated gold nanoparticles for the enhancement of glucose oxidase activity. *Langmuir* 23, 3333–3337. doi: 10.1021/la062901c
- Panigrahi, S., Basu, S., Praharaj, S., Pande, S., Jana, S., Pal, A., et al. (2007). Synthesis and size-selective catalysis by supported gold nanoparticles: study on heterogeneous and homogeneous catalytic process. *J. Phys. Chem. C* 111, 4596–4605. doi: 10.1021/jp067554u
- Pasternack, R. F., Gibbs, E. J., Collings, P. J., dePaula, J. C., Turzo, L. C., and Terracina, A. (1998). A non-conventional approach to supramolecular formation dynamics. *J. Am. Chem. Soc.* 120, 5873–5878. doi: 10.1021/ja980186q
- Pina, C. D., Falletta, E., and Rossi, M. (2011). Update on selective oxidation using gold. *Chem. Soc. Rev.* 41, 350–369. doi: 10.1039/C1CS15089H
- Qi, Y.-X., Zhang, M., Zhu, A., and Shi, G. (2015). Terbium (iii)/gold nanocluster conjugates: the development of a novel ratiometric fluorescent probe for mercury (ii) and a paper-based visual sensor. *Analyst* 140, 5656–5661. doi: 10.1039/C5AN00802F
- Qu, X., Sun, H., Zhou, Y., and Ren, J. (2018). Carbon nanozymes: enzymatic properties, catalytic mechanism and applications. *Angew. Chem.* 57, 9224–9237. doi: 10.1002/anie.201712469
- Quan, H., Zuo, C., Li, T., Liu, Y., Li, M., Zhong, M., et al. (2015). Electrochemical detection of carcinoembryonic antigen based on silver nanocluster/horseradish peroxidase nanocomposite as signal probe. *Electrochim. Acta.* 176, 893–897. doi: 10.1016/j.electacta.2015.07.086
- Roduner, E. (2006). Size matters: why nanomaterials are different. *Chem. Soc. Rev.* 35, 583–592. doi: 10.1039/b502142c
- Schaller, B., and Graf, R. (2004). Cerebral is chemia and reperfusion: the pathophysiologic concept as a basis for clinical therapy. *J. Cerebr. Blood. F. Met.* 24, 351–371. doi: 10.1097/00004647-200404000-00001
- Scopes, R. K. (2013). *Protein Purification: Principles and Practice*. LaTrobe, VIC: Spring Science Business Media.
- Senanayake, S. D., Stacchiola, D., and Rodriguez, J. A. (2013). Unique properties of ceria nanoparticles supported on metals: novel inverse ceria/copper catalysts for CO oxidation and the water-gas shift reaction. *Accounts. Chem. Res.* 46, 1702–1711. doi: 10.1021/ar300231p
- Sheldon, R. A. (2007). Enzyme immobilization: the quest for optimum performance. *Adv. Synth. Catal.* 349, 1289–1307. doi: 10.1002/adsc.200700082
- Shojaieard, Z., Hemmateenejad, B., and Shamsipur, M. (2016). Efficient on-off ratiometric fluorescence probe for cyanide ion based on perturbation of the interaction between gold nanoclusters and a copper (II)-phthalocyanine complex. *ACS Appl. Mater. Inter.* 8, 15177–15186. doi: 10.1021/acsami.6b01566
- Song, Y., Qu, K., Zhao, C., Ren, J., and Qu, X. (2010). Graphene oxide: intrinsic peroxidase catalytic activity and its application to glucose detection. *Adv. Mater.* 22, 2206–2210. doi: 10.1002/adma.200903783

- Spichiger-Keller, U. E. (2008). *Chemical Sensors and Biosensors for Medical and Biological Applications*. New York, NY: John Wiley and Sons.
- Steed, J. W., and Atwood, J. L. (2013). *Supramolecular Chemistry*. Durham: John Wiley and Sons.
- Tsipis, A. C., and Tsipis, C. A. (2003). Hydrometal analogues of aromatic hydrocarbons: a new class of cyclic hydrocoppers (I). *J. Am. Chem. Soc.* 125, 1136–1137. doi: 10.1021/ja028786j
- Tsunoyama, H., Sakurai, H., and Tsukuda, T. (2006). Size effect on the catalysis of gold clusters dispersed in water for aerobic oxidation of alcohol. *Chem. Phys. Lett.* 429, 528–532. doi: 10.1016/j.cplett.2006.08.066
- Velizarov, S., Crespo, J. G., and Reis, M. A. (2004). Removal of inorganic anions from drinking water supplies by membrane bio/processes. *Rev. Environ. Sci. Bio.* 3, 361–380. doi: 10.1007/s11157-004-4627-9
- Wan, Y., Yang, H., and Zhao, D. (2006). “Host–Guest” chemistry in the synthesis of ordered non-siliceous mesoporous materials. *Accounts Chem. Rev.* 39, 423–432. doi: 10.1021/ar050091a
- Wang, G.-L., Jin, L.-Y., Dong, Y.-M., Wu, X.-M., and Li, Z.-J. (2015). Intrinsic enzyme mimicking activity of gold nanoclusters upon visible light triggering and its application for colorimetric trypsin detection. *Biosens. Bioelectron.* 64, 523–529. doi: 10.1016/j.bios.2014.09.071
- Wang, X., Wu, P., Lv, Y., and Hou, X. (2011). Ultrasensitive fluorescence detection of glutaraldehyde in water samples with bovine serum albumin–Au nanoclusters. *Microchem. J.* 99, 327–331. doi: 10.1016/j.microc.2011.06.004
- Wang, X., Zhang, Y., Li, T., Tian, W., Zhang, Q., and Cheng, Y. (2013). Generation 9 polyamidoamine dendrimer encapsulated platinum nanoparticle mimics catalase size, shape, and catalytic activity. *Langmuir* 29, 5262–5270. doi: 10.1021/la3046077
- Wei, H., and Wang, E. (2013). Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. *Chem. Soc. Rev.* 42, 6060–6093. doi: 10.1039/c3cs35486e
- Wen, F., Dong, Y., Feng, L., Wang, S., and Zhang, S. (2011). Horseradish peroxidase functionalized fluorescent gold nanoclusters for hydrogen peroxide sensing. *Anal. Chem.* 83, 1193–1196. doi: 10.1021/ac1031447
- Wilhelmová, N. (1996). Cornish-bowden, a.: fundamentals of enzyme kinetics. *Biol. Planraum.* 38, 430–430. doi: 10.1007/BF02896674
- Wilson, R., and Turner, A.P.F. (1992). Glucose oxidase: an ideal enzyme. *Biosens. Bioelectron.* 7, 165–185. doi: 10.1016/0956-5663(92)87013-F
- Wu, J., Wang, X., Wang, Q., Lou, Z., Li, S., Zhu, Y., et al. (2019). Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem. Soc. Rev.* 48, 1004–1076. doi: 10.1039/C8CS00457A
- Xia, X., Long, Y., and Wang, J. (2013). Glucose oxidase-functionalized fluorescent gold nanoclusters as probes for glucose. *Anal. Chim. Acta.* 772, 81–86. doi: 10.1016/j.aca.2013.02.025
- Xiong, X., Tang, Y., Zhang, L., and Zhao, S. (2015). A label-free fluorescent assay for free chlorine in drinking water based on protein-stabilized gold nanoclusters. *Talanta* 132, 790–795. doi: 10.1016/j.talanta.2014.10.022
- Xu, B., Wang, H., Wang, W., Gao, L., Li, S., Pan, X., et al. (2019). A single-atom nanozyme for wound disinfection applications. *Angew. Chem. Int. Edit.* 58, 4911–4916. doi: 10.1002/anie.201813994
- Xu, X., Li, Y.-F., Zhao, J., Li, Y., Lin, J., Li, B., et al. (2015). Nanomaterial-based approaches for the detection and speciation of mercury. *Analyst.* 140, 7841–7853. doi: 10.1039/C5AN01519G
- Yan, R., Sun, S., Yang, J., Long, W., Wang, J., Mu, X., et al. (2019). A nanozyme-based bandage with single-atom catalysis for brain trauma. *ACS Nano.* 13, 11552–11560. doi: 10.1021/acsnano.9b05075
- Yang, B., Li, J., Deng, H., and Zhang, L. (2016). Progress of mimetic enzymes and their applications in chemical sensors. *Crit. Rev. Anal. Chem.* 46, 469–481. doi: 10.1080/10408347.2016.1151767
- Yang, X., Wang, J., Su, D., Xia, Q., Chai, F., Wang, C., et al. (2014). Fluorescent detection of TNT and 4-nitrophenol by BSA Au nanoclusters. *Dalton T* 43, 10057–10063. doi: 10.1039/C4DT00490F
- Yin, Y., and Talapin, D. (2013). The chemistry of functional nanomaterials. *Chem. Soc. Rev.* 42, 2484–2487. doi: 10.1039/c3cs90011h
- Yuwen, L., Xu, F., Xue, B., Luo, Z., Zhang, Q., Bao, B., et al. (2014). General synthesis of noble metal (Au, Ag, Pd, Pt) nanocrystal modified MoS₂ nanosheets and the enhanced catalytic activity of Pd–MoS₂ for methanol oxidation. *Nanoscale* 6, 5762–5769. doi: 10.1039/C3NR06084E
- Zhang, Y., Wang, F., Liu, C., Wang, Z., Kang, L., Huang, Y., et al. (2018). Nanozyme decorated metal–organic frameworks for enhanced photodynamic therapy. *ACS Nano.* 12, 651–661. doi: 10.1021/acsnano.7b07746
- Zhou, X., Xu, W., Liu, G., Panda, D., and Chen, P. (2010). Size-dependent catalytic activity and dynamics of gold nanoparticles at the single-molecule level. *J. Am. Chem. Soc.* 132, 138–146. doi: 10.1021/ja904307n
- Zhou, Y., Liu, B., Yang, R., and Liu, J. (2017). Filling in the gaps between nanozymes and enzymes: challenges and opportunities. *Bioconjugate Chem.* 28, 2903–2909. doi: 10.1021/acs.bioconjchem.7b00673
- Zhu, R., Zhou, Y., Wang, X.-L., Liang, L.-P., Long, Y.-J., Wang, Q.-L., et al. (2013). Detection of Hg²⁺ based on the selective inhibition of peroxidase mimetic activity of BSA-Au clusters. *Talanta* 117, 127–132. doi: 10.1016/j.talanta.2013.08.053

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2020 Zhang, Li, Liu, Long and Zhang. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.