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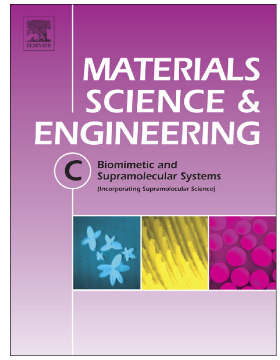

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Gold Nanozyme: Biosensing and Therapeutic Activities

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Abstract

The utilization of AuNPs in therapeutic applications has been accelerated by discovering their catalytic activity consistent with the activity of natural enzymes. However, to reduce unwanted activities, it is imperative to fully understand their catalytic mechanisms to increase efficiency and safety. Therefore, along with other reports, we aimed to classify the enzymatic activity of Au nanozymes based on recent advance in their applications in biosensing and therapeutic activities. The results of the reported experiments indicate that the Au nanozymes can be used in biosensing of a wide range of agents such as molecule (H_2O_2 and glucose), ions, nucleic acids, proteins, cells, and pathogens. Furthermore, they can be used as potential candidates in inhibition of neurodegenerative diseases, cancer therapy, and antibacterial activities. Biosensing and therapeutic activities are generally based on colorimetric assays and the controlling the ROS level in the targeted cells, respectively. Finally, a brief explanation of the current challenges of the Au nanozymes in biomedical approaches was discussed. Indeed, this review holds a great promise in understanding the Au nanozymes properties and their development in biotechnology, medicine, and related industries.

Keywords: Gold nanozymes, enzyme mimetics, biosensing, therapy

Abbreviation: Gold nanoparticle (AuNPs); Gold nanocluster (AuNCs); Graphene oxide (GO); Horse radish peroxidase (HRP); Limit of detection (LOD), Silver (Ag), Palladium (Pd), Platinum (Pt), Superoxide dismutase (SOD); Reactive oxygen species (ROS); Surface-enhanced Raman scattering (SERS); Surface plasmon resonance (SPR).

Contents

1. Introduction

AuNPs are well-studied nanomaterial, with potential applications in biomedical field due to their unique plasmonic features at the nanoscale [1, 2]. While Au does not present significant plasmonic characteristics in bulk form, they tend to show strong SPR features at the nanoscale [3-5]. Thus, the properties of AuNPs can be easily varied by changing their physicochemical properties [6]. Given this SPR property, AuNPs have received great attention for applications in different fields, especially in biomedicine, such as biosensors [7-9] and therapy [10, 11]. AuNPs also act as a promising agent for modifying detective imaging and selective therapy together for theranostic healing [12]. For biomedical application, AuNPs have been reported to be biologically inert as well as their physicochemical properties can be changed to obtain a safe nanozymes for reducing their side effects against normal tissues [13].

AuNPs represent one of the most important nanozymes [6, 14]. The analytical approaches used for Au and iron nanozymes characterization have been employed to understand other nanozymes [6, 14-17]. Furthermore, several research groups are currently using AuNPs as enzyme mimetics and have expanded the number of novel biomedical applications derived from their enzyme-like functions [17, 18]. Au nanozyme have been extensively used (Fig.1) in various fields, such as biosensing [19], immunological assay [20], cancer detection or treatment [21], drug delivery [22], nerve protection [23], stem cell growth [24] and removal of contaminants [25] based on their inherent catalytic properties. While several review papers regarding the general physical features of NPs have been published [6, 14-17, 26], only a few studies have focused on the intrinsic enzyme-like characteristics of AuNPs for biomedical applications [27-29]. In this review, we have systematically surveyed the latest advancements on Au nanozymes to move forward their application in biosensing and biomedical applications and draw the attention on the significance of Au nanozymes. The review also discusses the factors influencing the regulation of Au nanozymes in biosensing, cancer treatment and other medical applications, as well as the challenges and the development of Au nanozyme.

1.1. Facts

- AuNPs have been widely implemented in medical applications.
- AuNPs show promising enzyme-like activities which may cause the various applications of AuNPs in different biomedical settings.
- The variation in physicochemical properties of AuNPs can influence their biomedical responses.

1.2. Opening questions

- Could detection of molecules, especially those are important in medicine be achieved by the enzyme-like activity of Au nanozymes?
- Could nanozymes control neurodegenerative diseases based on the simulation of the activity of dismutase and peroxidase?
- Medical therapeutics and biosensing done by natural enzymes can be enhanced with the use of the Au nanozyme?
- Which mechanism would endow Au nanozymes with antibacterial activities?

2. Biosensing based on Au nanozymes

Common strategies for measuring molecules and biological compounds are the use of electrochemical, colorimetry and fluorescence biosensors among which the use of biosensors based on catalytic activity is more conventional. Since, the application of enzymes is expensive and complex in enzymatic biosensors, the use of compounds with the same enzymatic efficacy such as nanozymes has attracted much attention. The most important diagnostic biosensors based on nanozymes are including as following [30]:

2.1. Molecule sensing

Detection of molecules, especially H_2O_2 and glucose [30], because of their crucial roles in medicine is very vital. H_2O_2 detection is generally obtained by peroxidase mimicking activity of the Au nanozymes and the detection of glucose by the peroxidase- and the oxidase mimicking activity of Au nanozymes.

2.1.1. H_2O_2 detection

It is a fact that detection of H_2O_2 was performed by natural peroxidases including HRP) and Soybean peroxidase (SBP) in many fields of bioanalyses and pharmaceutical assays. However, several issues such as difficult purification, hard preparation, digestion and denaturation are accounted as the main disadvantages of peroxidases in industry. To combat these issues, nanomaterials was developed as the effectual alternatives of natural peroxidases [17, 31, 32]. In this line, AuNP sensors with positive charge were synthesized to detect H_2O_2 in an acidic buffer [33, 34]. Similarly, Han, et al. [35] with the generation of functionalized Au nanozymes containing positive charges, provided a simple, quick and sensitive approach for detecting H_2O_2 with a LOD of 0.06 mM in the linear range from 0.06 to 4.29 mM. It has also been shown that the use of nanocomposite containing Au nanozymes, AgNPs and GO, in addition to increasing the catalytic activity of Au nanozymes with a LOD of 1.26 nM, accelerated the peroxidase-like activity of Au nanozymes 2.8 times more than the natural enzymes [36]. At the same time, Wu, et al. [37] by designing an Au-Pt/SiO_2 platform-based colorimetric biosensor, not only increased the intrinsic peroxidase-like activity of Au nanozyme, but also provided it possible to detect H_2O_2 in human cervical cancer cells (HeLa)without toxicity (Fig. 2A). Likewise, Xu, et al. [38] by using Au-Pd alloy NPs decorated on quantum dots in GO, could detect H_2O_2 in real time in breast cancer tissue and increase the catalytic activity of Au nanozymes with a LOD of 500 nM and a linear range from 1.0 µM to 18.44 mM.

2.1.2. Glucose detection

Glucose can be detected by Au nanozymes when glucose oxidase is combined with a peroxidase-like nanozyme. This means that due to the activity of glucose oxidase, H_2O_2 is produced which allows the detection of glucose based on the peroxidase-like activity of AuNPs [39, 40]. In this regard, Deng, et al. [41] by applying N-acetyl-L-cysteine-protected AuNCs, designed a platform having a dual oxidase and peroxidase-like activity that was able to accurately detect serum glucose by non-invasive method with a LOD of 0.18 μM (the linear range was 0.39–27.22 μM) and a LOD of 0.002 U m/L (the linear range was 0.01–0.3 U m/L) for H_2O_2. Likewise, it has been shown that AuNPs@metal-organic framework (MIL)-101 nanozymes with oxidase and peroxidase mimicking activity along with plasmonic properties, in addition to the rapid detection of glucose and lactate in cardiovascular damage and ischemic stroke based on SERS, provides evaluating the efficacy and drug accumulation in the target tissue [42]. In the following, Vinita, et al. [43] with the development of a portable nanocomposite kit composed of AuNPs decorated over molybdenum disulfide quantum dots, in addition to increasing the stability of the kit under harsh conditions, improved glucose detection in blood, saliva and tear samples with a LOD of 0.068 μM (a linear range from 1 to 400 μM) compared to common sensors (Fig. 2B).

2.2. Ion sensing

Given that heavy metal ions such as Hg^{2+}, Pb^{2+}, Cd^{2+}, Co^{2+}, Pd^{2+} and Pt^{2+} are deleterious and toxic species in biological systems with significant oxidative damages and carcinogenic effects, development of a sensing system is necessary to facile detection of metal pollutions. Taking advantages of recent developments, AuNPs proposed as the colorimetric sensors to detect a wide variety of molecules like heavy molecules in living systems and environmental samples [44]. For instance, detection of Pb^{+} was also performed using AuNPs sensors based on colorimetric methods through functionalization of AuNPs with appropriate ligands as well as DNAzymes techniques in several systems [45, 46]. Likewise, DNA-AuNPs were used to detect Hg^{+2} based on DNA hybridization and ability of T-T mismatches to bind Hg^{2+} ions. As a result of T-T mismatch interaction with Hg^{2+} ions, a shift in T_m was observed and detection of Hg^{+2} was possible [47, 48]. In another method, amino acid homology and peptide-capped AuNPs sensors have been provided a colorimetric detection method to emerge Hg^{2+} and other heavy metal ions [49, 50]. In this line, the selective and sensitive methods were also described to trace Hg^{2+} in biological samples [51, 52]. In this field, the Au nanozyme-based paper chip (Au NZ-PAD) was presented for rapid detection of Hg^{2+} in aqueous systems [25]. Meanwhile, Chang, et al. [53] by developing the sensors of albumin-AuNCs, were able to identify Ag^{+} up to 0.204 μM by reducing the peroxidase-like activity of Au nanozymes. Analogously, it has been shown that the addition of Cu^{+} reduces the catalytic activity of the histidine-AuNCs, which can indicate the detection of Cu^{+} in the environment [54]. Recently, Deng, et al. [55] were able to detect a rare-earth elements such as Ce^{3+} using Au nanozymes based on the change in catalytic activity (Fig. 2C).

2.3. Nucleic acids sensing

Several studies have delineated that AuNPs in hybridation with nucleic acids and enzymes were able to detect DNA and/or miRNA [56]. Deoxyribozymes (DNAzymes) are known to identify genes and used in therapeutics [57]. For example, “10-25” DNAzymes by suppression of tumorigenesis genes in cancer cells seems to play a critical role in cancer therapy [58-61]. Despite the remarkable activity, DNAzymes unfortunately possess the problems of delivery and intracellular stability [62, 63]. In the light of this evidence, DNAzyme-AuNPs (DZNP) conjugates were synthesized to regulate gene expression, namely, 10-23 DZ-NPs were designed to modulate gene expression by cleavage of a target mRNA providing a new horizons in the development of therapeutic strategies [64]. Another interesting research by Liu and Lu [46] have been shown that DNAzyme-assembled AuNPs are a colorimetric Pb^{+2} biosensor with high selectivity implying the high potential of allostric DNA/RNAzymes (aptazymes) to detect a wide variety of analytes [65, 66]. Apart from these, Sun, et al. [67], synthesized a bimetallic Au_xPt_x nanozyme with a C-rich oligonucleotide core possessing peroxidase and thiophilicity mimicking activity which is able to quantitative detect bio-thioles including cysteine, homocystein and glutathione with high sensitivity in biological fluidity. Also, Liu, et al. [68] with the design of the Au nanozymes biosensors loaded on metal–organic frameworks (Fe-MIL-88), were able to detect single-stranded (ssDNA) and double-stranded DNA (dsDNA) with a LOD of 11.4 nM with a linear range of 30–150 nM based on the reduced peroxidase-like activity of the Au nanozymes. Another study was reported that intrinsic peroxidase-like activity of Au nanozymes could be multiplexed by inserting a ssDNA and/or RNA on a NP surface [69]. With this highly sensitive mechanism, detection of other analytes such as proteins can be provided using Au nanozymes-DNA conjugates with a LOD of 10 nM in urine samples (Fig. 3A) [70].

2.3.1. Aptamer sensing

In practice, aptamers are single stranded oligonucleotides with a length of nearly ten bases, high binding affinity and specificity to other molecules. Conjugates of aptamers with AuNPs (Apt-AuNPs) take advantages in early diagnosis and drug delivery. Meanwhile, Apt-AuNPs biosensors were evaluated in fluorescence, colorimetric, electrochemical and chemo luminescence sensing [71]. A useful application of aptamer-AuNPs was illustrated by Weerathunge, et al. [72] through the reversible inhibition of Au nanozymes using a 5-18 ssDNA aptamer for colorimetric detection of Acetamiprid as a potent neurotoxin among pesticides. In this regard, Hu, et al. [73] identified the level of Abrin with a LOD of 0.05 nM with a linear range of 0.2 nM to 17.5 nM based on the decrease in the peroxidase-like activity of the Au nanozymes. Furthermore, an enzyme-free assay was carried out for electrochemical detection of kanamycin in a highly sensitive and highly specificity manner [74]. It was also shown that the nanocomposite consisting of GO, Au/Pt NPs, aptamers (Apt15 and Apt29), and a G-quadruplex/hemin conjugate can accurately detect thrombin protein at a LOD of 0.15 nM with a linear range of 0.30 to 100 nM based on the change in the of peroxidase-like activity of Au nanozymes [75]. Whereas, Wu, et al. [76] showed that increasing Pt levels from below 0.5 to 2.5 % improved the performance of Au@Pt NPs in as enzyme mimicking properties and higher Raman signal stability up to 2 min. They also showed that increasing Pt amount to 25% reduced the enzymatic activity of the NPs. Recently, Das, et al. [77] using AuNPs containing the specific *Pseudomonas aeruginosa*–aptamer was able to detect bacteria based on the isolation of aptamer in the presence of *P. aeruginosa* and the peroxidase-like reactivity of Au nanozymes. Based on the mechanism of the separation of ssDNA aptamer from Au nanozymes in the presence of substrate, Tian [78], provided Hg^{2+} and microcystin-LR detection with a LOD of 3.63 nM and 7.14 ng/L with the linear range of 0.01–0.5 μM and 0.01–1.0 μg/L, respectively (Fig. 3B).

2.4. Protein sensing

One of the most essential techniques for protein detection is the use of an ELISA technique that nanozymes, especially Au nanozymes, can be an appropriate alternative as they show similar catalytic activity. For example, Gao, et al. [79] by designing a nanoprobe based on Au nanozymes containing peptide, increased the ability to detect integrin glycoprotein IIb/IIIa (GPIIb/IIIa) on the human erythroleukemia cells. This probe through the bio-conjugation method is attached explicitly to the integrin protein at the cyto-membrane surface and allows the examination of the integrin GPIIb/IIIa by changing the peroxidase and photoluminescence activity of the Au nanozymes. Similarly, based on the peroxidase-like activity of Au nanozymes loaded on nanoporous ferric oxide, detection of autoantibody p53 with a LOD of 0.08 U/mL was achieved in a cheaper and faster approach than common methods [80]. Furthermore, Zhang, et al. [81] using a Hemin-Au nanozymes@metal organic framework composite, in addition to increasing the peroxidase-like activity of Au nanozymes, detected alpha-fetoprotein (αFP) in blood with a LOD of lower than 0.02 ng/mL, which is also one of the biomarkers for diagnosis of cancer. At the same time, it has been shown that the presence of heparin proteins increases the catalytic activity of Au nanozymes [82]. Since, the presence of heparinase causes the breakdown of heparine and decrease peroxidase-like activity, heparinase and heparine can be detected by detecting a catalytic activity of Au nanozymes with a LOD of 0.06 μg/mL and a linear range of 0.1 to 3 μg/Ml (Fig. 3C) [82].

2.5. Cells and pathogens sensing

Excessively expressed protein at the cell surface as well as specific receptors in pathogens can be used as biological markers for the detection of cancers, bacteria or viruses. Hence, by using conventional techniques for detection of disease-related biomarkers which are complicated and time-consuming, innovative approaches based on Au nanozymes were evolved to shed light on colorimetric monitoring by naked eyes. In the colorimetric method, antibodies or biomarkers are generally immobilized on Au nanozymes or with other hybrids. Then, Au nanozymes attached to target cells and with intrinsic peroxidase-like activity in a solution containing 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) and 3,3′,5,5′-tetramethylbenzidine (TMB), by breaking the H_2O_2, causes a discoloration. In this line, bimetallic Au@Ag heterogeneous NRs were fabricated with the ability of peroxidase-like activity in neutral pH and assembly to form bio-nanostructures for detecting tumor cells and eventually, targeted therapy [27, 83]. Also, Liu, et al. [84] with the design of the Au-iron oxide sensors based on colorimetry, in addition to increasing the catalytic capability of the Au nanozymes, detected HeLa cancerous cells with a LOD of fewer than 100 cells. It was also found that the GO-silicon-folic acid@AuNPs hybrid, based on the peroxidase-like activity of Au nanozymes, in addition to therapeutic activity through increasing $OH^•$ radical, is a fast probe for the detection of HeLa cells with a LOD of fewer than 50 cells and a linear range of 1 to 1×10^5 cells [85]. Likewise, Tao, et al. [86] with the development of colorimetric biosensors based on the peroxidase-like activity of AuNCs, not only accelerated the detection of breast cancer according to HER2 biomarker in human serum rather than current sensors, but also increase the LOD of breast cancer to 5 cells with a linear range of 5 to 1000 cells (Fig. 3D).

On the other hand, AuNPs with peroxidase-like activity were proposed to detect avian influenza virus antigens. In this field, Ahmed, et al. [20] obtained a higher LOD (1.11 pg/mL) than the ELISA method by attaching Au nanozymes to the anti-HA H5N1 antibody. Furthermore, a highly monodispersed Au nanobipyramids with a Ag shell with Ag ion and 4-aminophenol was developed to detect ALP and H5N1 influenza virus antigen [87]. Surprisingly, semi-quantitative detection of other biomarkers is possible with naked eye in this colorimetric assay. For instance, Khoris, et al. [88] with the development of anti-nov genogroup II antibodies-containing kits immobilized on Au/Ag NPs, in addition to increasing the detection of the norovirus (10.8 pg/mL) between 100 to 1,000 folds more than ELISA method, enhance the rapid detection of the virus based on the enhancement of the activity of peroxidase-like activity of Au nanozymes during the presence of norovirus.

3. Therapeutic activities based on Au nanozymes

Because, controlling reactive nitrogen and oxygen species should be used in medical activities according to biomedical models; researchers believe that biocompatible and biodegradable nanozymes, especially iron and Au nanozymes, can be used to control these species with the oxidase, peroxidase and even dismutase-like activities. Therefore, the therapeutic effects of Au nanozymes are discussed in this section.

3.1. Inhibiting neurodegenerative diseases

Because, the activity of peroxidase and dismutase is crucial in controlling neurological diseases, especially Alzheimer's disease, researchers have been inspired by natural enzymes to develop nanozymes for controlling neurodegenerative diseases based on the simulation of the dismutase and peroxidase-like activity of NPs[89]. In general, the main route of treatment and prevention of Alzheimer's disease is to control the amyloid-β protein aggregation and ROS in the brain tissue. Also, reducing the copper accumulation in the brain is very effective in controlling the neurodegenerative diseases. The results of Gao, et al. [90] suggested that the use of Au nanozymes@polyoxometalate-8peptide nanocomplex reduces the ROS based on the SOD-like activity of Au nanozymes and the aggregation of amyloid-β protein according to the protease-like activity of the polyoxometalate (Fig. 4A). It was also shown that Au nanozymes act as a metal chelator and collect Cu from the Aβ oligomers. As a result, the side effects of Cu accumulation by inducing toxicity and subsequent protein accumulation, are reduced [90].

3.2. Cancer therapy

Because, excessive production of ROS can damage cancer cells, nanozymes can increase ROS levels in cancer cells by applying oxidase and peroxidase-like activities of NPs. For example, Maji, et al. [85] using GO-silicon-folic acid@AuNPs hybrid based on the intrinsic peroxidase-like activity, were able to provide therapeutic activity by increasing the $OH^{\bullet}$ and toxicity in HeLa cells. While, they revealed that the use of GO-silicon-folic acid@AuNPs hybrid in healthy cells did not lead to cytotoxicty. This potential approach represents a smart treatment for cancerous cells. Also, localized hypoxia in solid tumors cause's problems in treating cancer through radiotherapy, which Yi, et al. [91] by using Au nanozymes@manganese dioxide NPs, could increase the oxygen in the radiotherapy-resistant hypoxia tumor tissue despite reducing the toxic effects of NPs into other tissues. Also, it has been well documented that optical activities such as photodynamic or photothermal therapy, can induce a synergetic effect in increasing ROS production [92]. For example, in the treatment of drug-resistant cancers, the efficacy of treatment is greatly enhanced by the use of PAMAM dendrimer-encapsulated AuNCs (AuNCs-NH_2) and photodynamic therapy to produce oxygen through increased catalase activity in cancerous tumors [93]. The AuNCs-NH_2 not only increases the consumption of H_2O_2 optimally by catalase to produce oxygen, but also maintains the catalase activity in the development of acidic condition, even up to pH=4. Subsequently, Fan, et al. [94] by designing a nanostructure core (Au nanozymes)-shell (porous hollow carbon nanospheres) for the production of ROS through the simulation of the peroxidase and oxidase-like activities of Au nanozymes and its application in human and animal cellular studies, could significantly reduce the activity of cancerous cells (Fig.

4B). Also, they described that the use of the photothermal method increases the Au@HCNs performance in ROS production and mortality of the tumorous cells.

3.3. Antibacterial activity

Au nanozymes show pH-switchable peroxidase and catalase-like activities. As a result, these compounds could exhibit a potent antibacterial activity by producing ROS in a defined pH. In this line, it was found that Au nanozymes immobilized on mesoporous silica with peroxidase-like activities can eliminate Gram-positive bacteria (*Staphylococcus aureus*) and Gram-negative bacteria (*Escherichia coli*) under physiological condition based on the production of $^1O_2$, $OH^{\bullet-}$, and $O_2^{\bullet-}$ in an acidic pH [95]. Analogously, Wang, et al. [96] by designing a sheet of Au nanozymes and ultrathin graphitic carbon nitride, were able to accelerate wound healing not only by killing Gram-negative and Gram-positive bacteria through $OH^{\bullet-}$ production, but also exposing high efficiency in breaking biofilm creatures and preventing the formation of new biofilms. Recently, in a mice model, it has been reported that the use of Au-Ag alloy with isonicotinyl hydrazide based on ROS generation can kill the drug-resistant M. *tuberculosis*, despite its low toxicity for cells based on MTT and LDH tests (Fig. 4C) [97].

4. Challenges and future perspective

Since, NPs have been explored as a nanozyme, many researchers have drawn attention to NPs due to low cost of production, easy preparation, stability, and excellent durability in hard conditions than natural enzymes. Although, nanozymes are widely used as biosensing, antibacterial, anti-cancer and antioxidants agents, there are some crucial challenges as following:

1- Because, Au nanozymes are generally produced through test and error strategies, in order to increase their biomedical performance, we need to have a better understanding the catalytic mechanisms in both the empirical and computational fields. Also, attention to concepts of active site and its specificity should be considered.

2- Despite the extensive work of the researchers to enhance the selectivity and specificity of NPs, there is not enough information on Au nanozymes. For this purpose, it is necessary to consider the rate of enzymatic activity, selectivity, sensitivity, and higher productivity of Au nanozymes.

3- Although, the main catalytic activity of the Au nanozymes is nearly ascertained, yet not all of their possible enzymatic reactions have been mentioned. For this purpose, in addition to increasing the experiments *in vitro* and *in vivo*, an appropriate method for combining the catalytic activity of Au nanozymes in a single unit is necessary to provide a reasonable prediction of the unwanted reactions and its possible effects.

4- In order to increase the potential effects of the enzyme-like activity of Au nanozymes, all promising impacts of Au nanozymes in chemistry and biology should be provided.

5- Due to the multifunctional use of AuNPs in biosensing and therapeutic processes, it is pivotal to explore the probability of using Au nanozymes in multi-purpose activities. Probably, the simultaneous utilization of magnetic, thermal and optical properties of Au nanozymes can optimize their application in biosensing and biomedical fields.

5- To advance Au nanozymes in therapeutic applications, all of their potential benefits and risks about cellular processes, clinical toxicity, immunogenicity, pharmacokinetics, and so on should be investigated. Also, their possible combination with receptors, substrate-like compounds and intermediate compounds of biochemical pathways in the long term should be considered.

6- Researchers need to consider the performance of Au nanozymes in the presence of organic and non-organic nanozymes. The appearance of new properties due to the combination of nanozymes can lead to occurrence of a synergic response or multiple reactions simultaneously, which will be the solution to many health problems.

5. Conclusions

Due to the proper biocompatibility of Au nanozymes and the ability to use them in various activities such as biosensing, and therapeutic applications, their development as part of an artificial enzyme would be very appropriate. Most reported studies relate to the catalytic activity of Au nanozymes *in vitro*, whereas *in vivo* reports are more important for potential application of AuNPs in therapeutic activities. Therefore, providing a brief overview in this article can provide a new avenue in the optimization of enzymatic-like activities of Au nanozymes *in vivo*. Eventually, future developments in Au nanozymes will lead to the formation of new biocatalysts based on past information and challenges ahead. Thus, in this paper, in addition to presenting important reports in this field, we tried to address their challenges.

Conflicts of interest

The authors have none to declare.

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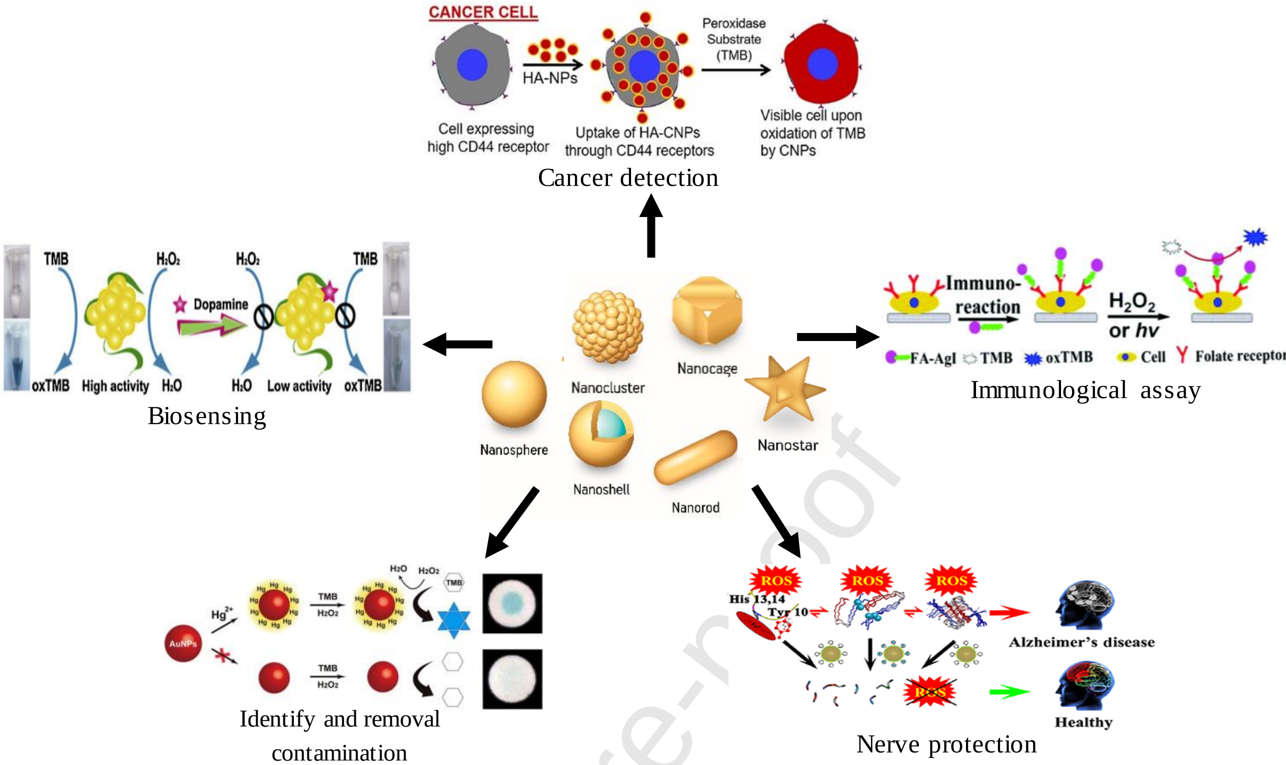

Figure 1. The application of Au nanozymes in different fields.

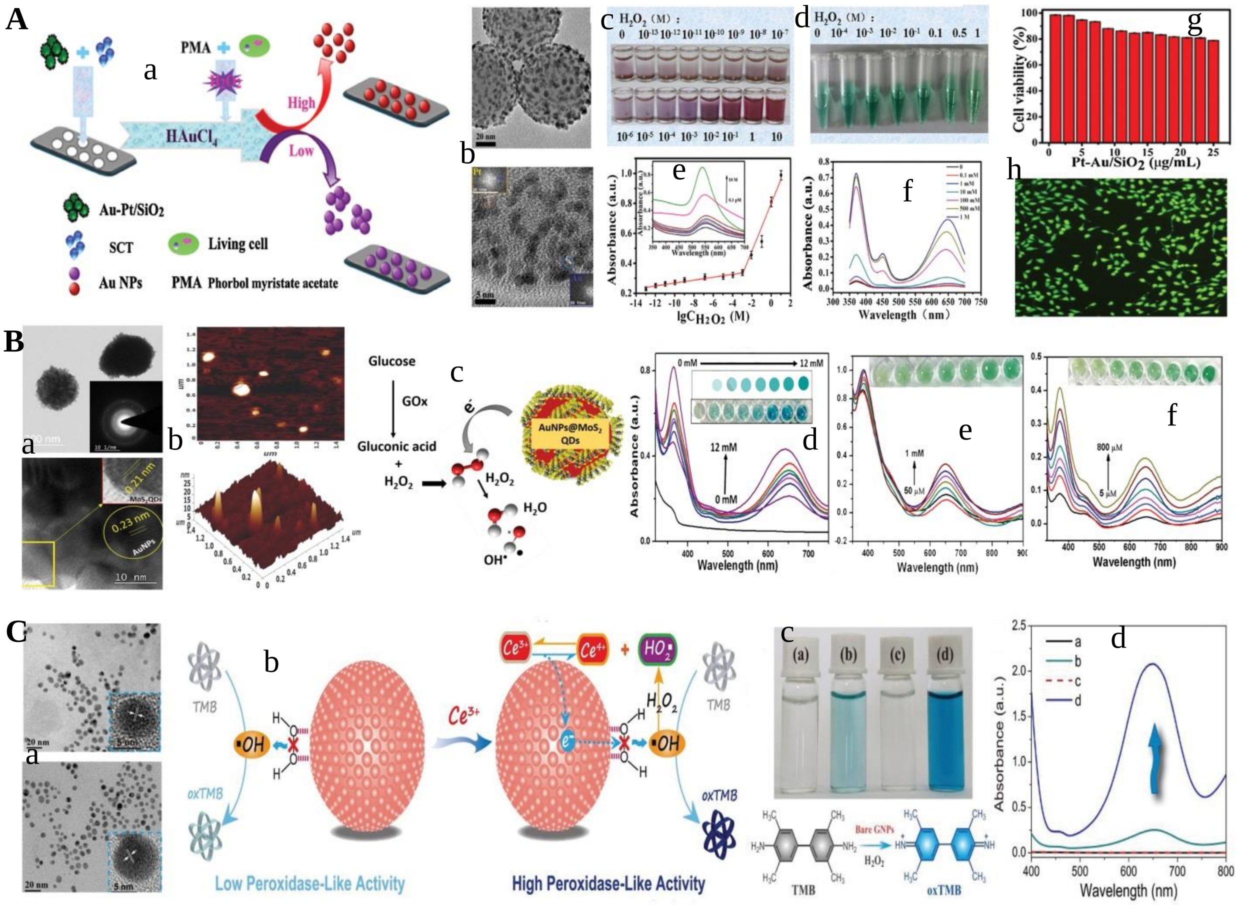

Figure 2. Molecule and ion sensing. (A): Detection of intracellular hydrogen peroxide. a; schematic illustration of the direct reduction of $HAuCl_4$ for the visual detection of intracellular H_2O_2. b; a magnified version of Au-Pt/Si NPs for above plan and HRTEM image of Au-Pt/Si NPs for under plan. c; colorimetric detection of H_2O_2 with the proposed $HAuCl_4/H_2O_2$ system and, d; TMB/H_2O_2 system in the presence of APS NPs. d; calibration curves of UV–vis absorbance vs different H_2O_2 concentrations on a logarithmic scale. e; the corresponding UV–vis absorption spectra of TMB–H_2O_2 reaction system (TMB: 0.6 mM) with different concentrations of H_2O_2 in the presence of Au-Pt/Si NPs. g; relative viability of HeLa cells incubated with a series of gradient concentrations of Au-Pt/Si NPs (1.0–25 g/mL). h; fluorescence imaging of HeLa cells cultured with Au-Pt/Si NPs for 24 h [37]. **(B): Peroxidase mimetic for detection of glucose.** a; TEM image of AuNPs@MoS_2-QDs composite. b; AFM image of AuNPs@MoS_2-QDs. c; the catalytic mechanism of TMB oxidation in presence H_2O_2 catalyzed by AuNPs@MoS_2-QDs. d; UV-Vis spectra of test of glucose level in serum by portable test kit and inset change the color of wells hydrogel with presence glucose level, with typical colorimetric chart with level glucose (in order to 0, 2, 4, 5, 8, 10, 11, 12 mM). e and f; absorption spectra of test of glucose level in tear by portable test kit and inset change the color of wells hydrogel with presence glucose level (50, 100, 250, 400, 600, 800 μM and 1 mM) in tear and saliva [43]. **(C): Ultrasensitive colorimetric detection of rare earth Ce^{3+} ion.** a; TEM images of the bare AuNPs before (above plan) and after (under plan) treatment with 200 nM Ce^{3+}. Inset: HRTEM image of the bare AuNPs. b; illustration of the Ce^{3+} enhanced peroxidase-like activity of bare AuNPs. c; photographs of (a) 0.25 M H_2O_2+0.4 mM TMB, (b) bare AuNPs+0.25 M H_2O_2+0.4 mM TMB, (c) 200 nM Ce^{3+}+0.25 M H_2O_2+0.4 mM TMB, and (d) bare AuNPs+200 nM Ce^{3+}+ 0.25 M H_2O_2+0.4 mM TMB; Bottom: bare GNP-catalyzed reaction of TMB with H_2O_2. d; UV-vis absorption spectra of (a) 0.25 M H_2O_2+0.4 mM TMB, (b) bare AuNPs+0.25 M H_2O_2+0.4 mM TMB, (c) 200 nM Ce^{3+}+0.25 M H_2O_2+0.4 mM TMB, and (d) bare AuNPs+200 nM Ce^{3+}+0.25 M H_2O_2+0.4 mM TMB [55].

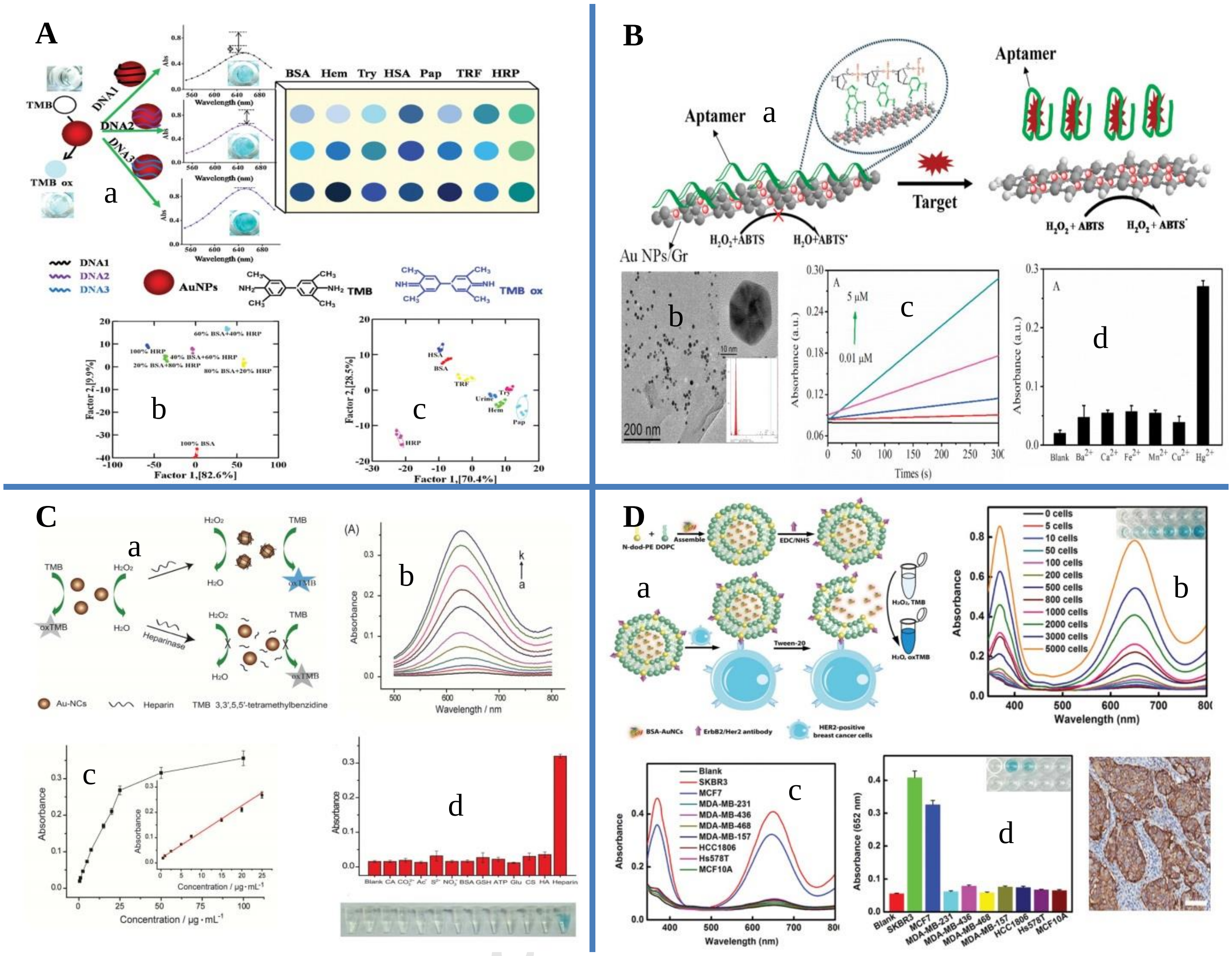

Figure 3. Biosensing based on AuNPs. (A): Colorimetric sensor array for proteins discrimination. a; schematic illustration of proteins recognition by using colorimetric sensor array based on enzymatic amplification of AuNPs absorbed with different single-stranded DNA. b; canonical score plot for discrimination mixtures of BSA and HRP at different molar ratios at total protein concentration of 50 nM. c; canonical score plot for the discrimination of proteins at the 100 nM levels picked in human urine by using the colorimetric sensor array based on AuNPs-DNA catalytic reaction in the presence of substrate TMB and H_2O_2 [70]. **(B): Aptamer-based colorimetric detection.** a; schematic illustration of the colorimetric determination of targets based on the peroxidase-like activity of AuNPs/GO. b; TEM image of AuNPs/GO (inset are the TEM image and EDX spectrum of the AuNPs in AuNPs/Gr). c; time-dependent absorbance changes of ABTS oxidation solution with H_2O_2 catalyzed by AuNPs/GO at various concentrations of Hg^{2+} (0.1, 0.5, 1, 3, and 5 µM). d; effects of coexisting ions on Hg^{2+} detection. The final concentrations of Hg^{2+} and interferences were 5 µM [78]. **(C): Peroxidase-like activity of Au nanoclusters for colorimetric detection of heparin.** a; schematic illustration of the method for heparin and heparinase detection. b; absorption spectra of the TMB/H_2O_2/Au-NCs system in the presence of different heparin concentrations (from a to k: 0, 0.5, 1, 2, 2.5, 7.5, 15, 20, 25, 50, 100 µg/mL). c; plots of the absorbance of the system at 652 nm versus the heparin concentrations. Inset: linear plot of heparin, each point is the average of three measurements. d; the absorbance value and corresponding photograph of the catalytic oxidation of TMB-H_2O_2 by

Au-NCs in the presence of various anions and biomolecules. Concentrations: citric acid, CO_3^{2-}, Ac^-, S^{2-}, NO_3^-, GSH, ATP, glucose, 10 mM; BSA, CS, HA, Heparin, 100 µg/mL [82]. **(D): Colorimetric sensor for HER2-positive breast cancer cell detection.** a; schematic representation of preparation of anti-HER2 conjugated liposome-AuNCs hybrid (BSA-AuNCs-LPs-anti-HER2) and HER2-positive breast cancer cell detection by using BSA-AuNCs-LPs-anti-HER2. b; the absorbance spectra and visual color changes upon analyzing different number of SKBR3 cells. c-d; selectivity analysis for HER2-positive breast cancer cell detection using BSA-AuNCs-LPs-anti-HER2 by monitoring the absorbance spectra and color changes [86].

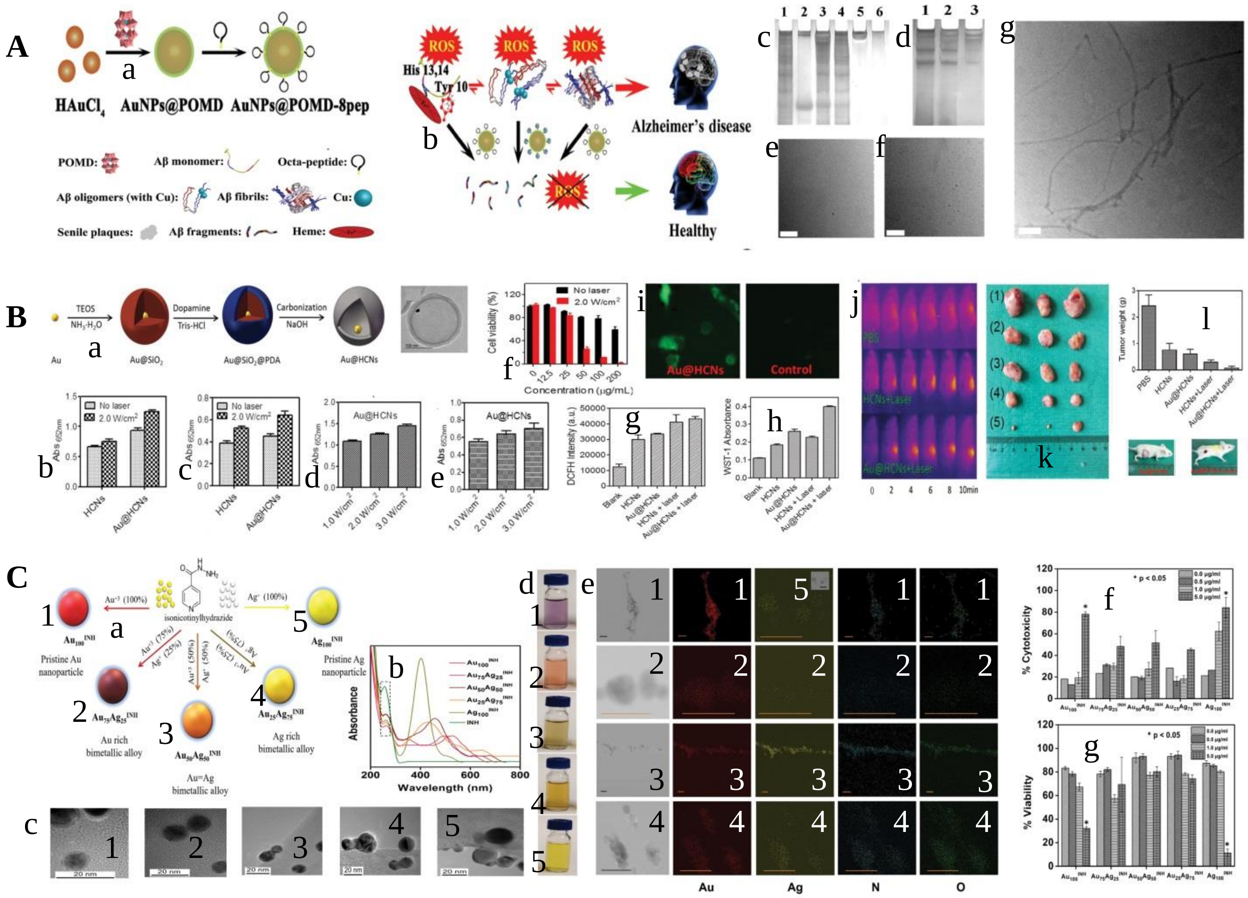

Figure 4. Therapeutic activity based on AuNPs. (A): Polyoxometalate-based nanozyme for treatment of Alzheimer's disease. a; synthetic route of the nanozyme. b; AuNPs@POMD-8pep acted as a multifunctional nanozyme to modulate multiple facets of Alzheimer's disease. c; determination of the AuNPs@POMD-8pep on the formation of Aβ fibrils by native PAGE. Lane 1: control 1 (Aβ 1-40 after incubation for 7 days); lane 2: control 2 (Aβ monomer only); lane 3: Aβ1-40/AuNPs@POMD after incubation for 7 days; lane 4: Aβ1-40/8pep after incubation for 7 days; lane 5: Aβ1-40/AuNPs@POMD-8pep after incubation for 7 days; lane 6: the supernatant in lane 5 (nanozyme removed) continuously incubated for an additional 7 days. All incubations were performed at 37 °C. d; determination of the AuNPs@POMD-8pep on the hydrolysis of Aβ preformed fibrils by native PAGE. Lane 1: control 1 (Aβ 1-40 after incubation for 7 days), lane 2: Aβ preformed fibrils/ AuNPs@POMD-8pep for 1 day, lane 3: Aβ preformed fibrils/ AuNPs@POMD-8pep for 3 days. e-f; the hydrolyzing effect of AuNPs@POMD-8pep on Aβ preformed fibrils was determined by TEM. Scale bars equal 100 nm. All incubations were performed at 37°C. g; Aβ fibrils visulaized by TEM [90]. **(B): Tumor catalytic-photothermal therapy.** a; schematic diagram of Au@HCNs synthsis and TEM images of Au@HCNs. b-d; peroxidase-like activity (reaction time: 3 min, laser irradiation time: 1 min); c-e; oxidase-like activity (reaction time: 30 min, laser irradiation time: 5 min). f; the power of NIR was 2.0 W/cm^2. Relative viabilities of CT26 cells incubated with Au@HCNs with and without 808-nm laser irradiation for 10 min. g; fluorescence intensity of DCFH determining the concentration of ROS (808-nm, 3min). h; absorbance of WST-1 showing the presence of superoxide in cells (808-nm, 3min). The concentrations of HCNs and Au@HCNs were both 50 μg/mL. The power of

NIR was 2.0 W/cm^2. i; fluorescence images of cells treated with Au@HCNs and control. j; IR thermal images of CT26 tumor-bear mice with the NIR laser irradiation (808 nm, 2.0 W/cm^2, 10 min) after intravenous injection with PBS,HCNs and Au@HCNs. k; the body weight after various treatments during 21 days. Photos of tumors from (1) control, (2) HCNs, (3) Au@HCNs, (4) HCNs+Laser, (5) Au@HCNs+Laser. l; the tumor weight after various treatments indicated over 21 days [94]. **(C): biocompatible bimetallic alloy NPs of Au and Ag**. a; illustration of single step synthesis of mono and bimetallic alloy NPs of Au and Ag using iso-nicotinyl hydrazide (INH) as reducing and stabilizing agent. b; absorbance spectra of INH-mediated Au, bimetallic Au-Ag alloys, Ag NPs and INH. c; TEM micrographs of (1-5) Au_{100} INH, $Au_{75}Ag_{25}$ INH, $Au_{50}Ag_{50}$ INH, $Au_{25}Ag_{75}$ INH and Ag_{100} INH NPs. d; digital photographs of (1-5) Au_{100} INH, $Au_{75}Ag_{25}$ INH, $Au_{50}Ag_{50}$ INH, $Au_{25}Ag_{75}$ INH and Ag_{100} INH NP solutions synthesized using INH. e; elemental mapping images obtained by STEM-EDS of Au_{100} INH (1), $Au_{75}Ag_{25}$ INH (2), $Au_{50}Ag_{50}$ INH (3), $Au_{25}Ag_{75}$ INH (4) and Ag_{100} INH (5). The scale bar in all micrographs represents 50 nm. g; LDH assay to determine the released amount of lactate dehydrogenase as a result of cytotoxicity; h; fibroblast cells viability determined by 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyltetrazolium bromide assay [97].

Highlights

Biosensing based on gold-nanozymes

Molecule sensing

Ion sensing

Nucleic acids sensing

Protein sensing

Cells and pathogens sensing

Therapeutic activities based on gold-nanozymes