Noble-metal nanocluster as enzyme-mimetic catalyst for diagnostic analysis

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Received August 20, 2019; accepted October 8, 2019; published online October 24, 2019

Citation: Ren X J, Wang S Z, Su D D, et al. Noble-metal nanocluster as enzyme-mimetic catalyst for diagnostic analysis. *Sci China Tech Sci*, 2019, 62: 2306–2309, <https://doi.org/10.1007/s11431-019-1459-3>

Noble-metal nanoclusters, which consist of only several to a few hundred metal atoms, have attracted a great deal of scientific interest due to their fascinating optical properties, biocompatibility and low toxicity [1]. Intriguingly, nanoclusters have been recently reported possessing intrinsic enzyme-mimetic properties, mainly including peroxidase-like activity, which can convert hydrogen peroxide into hydroxyl radicals, and catalase-like activity, which can convert hydrogen peroxide into water and oxygen. As low-cost alternatives to natural enzymes, nanoclusters can overcome several drawbacks of natural enzymes, such as sensitivity of catalytic activity to environmental conditions, low stability led by denaturation and digestion and high cost. In comparison to other nanomaterial based enzyme mimetics, nanoclusters are more prominent for bioanalysis due to their ultrasmall size, accurate molecular formula and excellent stability [2]. Diagnosis applications have recently started to emerge for nanoclusters with enzyme-mimetic properties, including biomarker detection, bio-imaging, protection against neurodegeneration and tumor prevention (Figure 1) [3].

Bovine serum albumin (BSA) stabilized Au nanoclusters (Au NCs) have firstly been found to possess highly intrinsic peroxidase-like activity, which can convert hydrogen peroxide into hydroxyl radicals. Consequently, most of reports for analyte detection are based on hydrogen peroxide detection by combining peroxidase mimicking nanoclusters

with a reporter substrate such as 3,3',5,5'-tetramethylbenzidine (TMB) or luminol. This process can achieve the naked eye readout, which offers outstanding advantages of low cost, simple operation process, lack of requirement for complicated equipment, and easy-to-use applications. This colorimetric method can meet the need of on-site and read time detection, therefore hold potential applications for colorimetric sensors in biological and diagnosis areas. Based on the BSA-Au NCs as peroxidase mimetics, Wang et al. [4] developed a colorimetric method for xanthine detection. By combining intrinsic peroxidase-like activity and fluorescence properties of BSA-Au NCs, Tao et al. [5] developed a dual colorimetric and fluorometric sensor for highly sensitive and selective detection of dopamine in real samples. In addition, other noble-metal nanoclusters have also been found to possess intrinsic peroxidase-like activity, such as BSA stabilized copper nanoclusters (Cu NCs) [6], yeast extract stabilized Pt nanoclusters (Pt NCs) [7], monothiolated β -cyclodextrin stabilized Cu NCs [8], DNA-templated Ag/Pt bimetallic nanoclusters (DNA-Ag/Pt NCs) [9] and so on. Some have been developed as highly sensitive colorimetric sensors for glucose or thrombin detection. Recently, amino acid stabilized AuNCs have been reported to have similar peroxidase-mimic activity like protein stabilized AuNCs. Amino acids are remarkable for their ambidentate nature and capability of forming stable complexes with various metal ions. Based on histidine's ambidentate character, Liu et al. [10] rationally designed histidine stabilized AuNCs for ultrasensitive col-

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Figure 1 (Color online) Noble-metal nanocluster as enzyme-mimetic catalyst for diagnostic analysis.

orimetric assay of copper ions (Cu^{2+}) and histidine. The peroxidase-like activity of His-AuNCs can be efficiently inhibited in the presence of Cu^{2+} . Then upon addition of histidine into His-AuNCs/ Cu^{2+} system, the chelation between the imidazole group of histidine and Cu^{2+} leads to Cu^{2+} release from His-AuNCs, and results in a significant recovery of enzyme activity of His-AuNCs.

Subsequently, nanoclusters have been applied for immunoassays such as ELISAs or capture-detection assays via conjugating antibodies or other targeting ligands. For example, based on a sandwiched antibody-antigen (or other analytes)-antibody structure, Zhao et al. [11] conjugated enzyme-mimetic Au NCs on the outer layer antibody to catalyze hydrogen peroxide decomposition which was used to reduce HAuCl_4 into gold nanoparticles for naked eye readout. This plasmonic nanosensor demonstrated high applicability for a variety of ultratrace analytes such as protein avidin, thyroid hormone, cancer antigen, and methamphetamine (MA), reaching ultrahigh sensitivity, for instant, nearly single molecular detection of MA.

Owing to their small size which is one or more orders of magnitude smaller than cells, nanoclusters can be taken up by cells or penetrate into tissues by passing between cells and diffusing through the extracellular matrix. Thus nanoclusters exhibit a clinical potential for bioimaging, cancer diagnosis and therapy [12,13]. Since folate receptors are under-expressed in normal tissues and overexpressed on the surface of different types of cancer cells, Hu et al. [14] developed a novel fluorescence enzyme mimetic nanoprobe based on folate receptor-targeting AuNCs. The nanoprobe could achieve peroxidase staining and fluorescent staining of the same tumor tissue slice simultaneously, and the results were mutually complementary. Nevertheless, the potential of AuNCs as enzyme mimic has also been limited by the low catalytic activity at neutral pH. Remarkably, mental nanoclusters can be flexibly engineered to modify their prop-

erties and boost catalytic activity. Currently, it is reported that graphene oxide (GO) could serve as the enzyme modulator to regulate the peroxidase-like activity of lysozyme stabilized AuNCs [15]. GO-AuNCs hybrid exhibits high catalytic activity over a broad pH range, even at neutral pH. As GO possesses high surface-to-volume ratios and high affinity for hydrophobic molecules, it could bind to substrate and catalyst in the same nanoscale region, which could greatly enhance the catalytic activity of AuNCs. Qu's group conjugated folic acid on GO-AuNCs hybrid and developed a fast, cheap, and selective colorimetric approach to detect cancer cells at physical pH. Inspired by these favorable results, our group has also synthesized peptide stabilized AuNCs for cancer cell detection [16]. The peptide can be designed to specifically target Membrane type 1 matrix metalloproteinase (MT1-MMP) which is positive regulators of tumor development, invasion and metastasis. By utilizing the intrinsic peroxidase-like activity of the AuNC probes, we can achieve assessing the expression level of MT1-MMP on the SH-SY5Y cells by the naked eye without cell lysis and protein extraction process. Meanwhile, Au count determined by inductively coupled plasma mass spectrometry (ICP-MS) and fluorescence intensity were acquired to verify the feasibility of this method. More importantly, due to the accurate molecular formula of peptide-cluster probe, we combined laser ablation inductively coupled plasma mass spectrometry (LA-ICP-MS) technique for single cell Au count determination, thus achieved precise quantification of membrane proteins expression level on single cells [17].

The emergence of the enzyme-mimetic properties of these nanoclusters has created new and exciting therapeutic opportunities beyond the traditional biomedical applications as carriers or for their optical properties. Recent works have demonstrated that mental nanoclusters can maintain catalytic processes under physiological conditions and provide therapeutic activity. Our group has explored peptide-coated Au NCs to selectively enhance the tumor-specific oxidative pressure with high efficiency [18]. By rationally designing peptide sequences to target integrin $\alpha_v\beta_3$ which was over-expressed on glioblastoma cells, the peptide-coated AuNCs can selectively recognize integrin on tumor cells and enter the cells. Then it catalyzes H_2O_2 to produce higher-toxic superoxide anion radicals ($\text{O}_2^{\cdot-}$) and leads to the obvious enhancement of the reactive oxygen species (ROS) level in tumor cells, which thus induce mitochondria-dependent apoptosis and achieve high efficient anticancer therapy [19]. Lin and their colleagues [20] have reported for the first time that amine-terminated PAMAM dendrimer-entrapped gold nanoclusters (AuNCs-NH_2) lose their peroxidase-like activity unexpectedly while still retaining catalase-like activity in physiological conditions. The reason was proposed that the enrichment of polymeric 3°-amines on the surface of AuNCs-NH_2 significantly suppressed the pivotal mediator

•OH for the peroxidase-like activity. Due to the inhibition of intrinsic peroxidase-like activity, AuNCs-NH₂ was conferred with diminished cytotoxicity, which therefore show huge potential for safe and long-term use. As ROS are the fundamental mediators of neurotoxicity in ischemia or reperfusion injury [21], the remained catalase-like activity of AuNCs-NH₂ was used for neuroprotection without enzyme encapsulation and controllable release. It is indicated that AuNCs-NH₂ treatment show an efficient suppression of intracellular H₂O₂, which protecting neurons against H₂O₂-induced toxicity. By making use of this exciting feature, their group further employed AuNCs-NH₂ for photodynamic therapy [22] (PDT) against hypoxia of cancer cells. AuNCs-NH₂ can self-supply O₂ for PDT because that their intrinsic catalase-like activity can catalyze physiological H₂O₂ to generate O₂. Importantly, attributed to their surface of dendrimers, AuNCs-NH₂ extended the catalase-like activity to acidic conditions. They found that sequential treatments of hypoxic cancer cells with AuNCs-NH₂ and photosensitizers before PDT significantly enhanced the PDT efficacy [23]. This study holds great promise for potential applications such as PDT to achieve more effective anticancer treatments.

Despite exciting progress in the development of metal nanoclusters with enzyme-mimetic properties in diagnostic applications, there are still considerable hurdles and challenges remaining with respect to promoting the performance of metal nanoclusters. Firstly, nanoclusters usually exhibit relatively low catalytic activity compared with natural enzymes. Changing the functional groups in the catalytic site or designing nanomaterials cores will be promising means to obtain high catalytic performance. Alternatively, exploring efficient modulators can also tailor the catalytic activities of these metal nanoclusters. Another important issue is that most of the work using catalytic nanoclusters has been performed prevalently *in vitro* without taking account of the complexity of biological systems. Enzymatic properties and kinetics of nanoclusters can be affected by their micro-environment [24] and are challenging to control. The surface chemistries of nanoclusters can also be changed in the presence of biological fluids. It will be more valuable to investigate factors which affect catalytic activity and long-term effects of nanoclusters in biological systems. For example, developing new biomolecule-templated metal nanoclusters or increasing the surface-to volume ratio and the population of low-coordination sites, will be favorable for enhancing *in vivo* compatibility and broadening theranostic applications. In addition, these nanoclusters are limited to catalyze only two types of reactions, peroxidase-like and catalase-like reactions, whereas natural enzymes are known to catalyze a wide range of important biochemical reactions. Therefore, exploring the potential of nanoclusters for catalyzing new types of reactions are indeed essential. The field of nanoclusters enzyme mimetics is still in its infancy. Future

directions should be centered on achieving maximal efficacy, stability and specificity with long-term effects and minimal toxicity as well as developing cost-effective formulations, which would be critical for application in clinical diagnostics.

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