Fluorescence Quenchers Manipulate the Peroxidase-like Activity of Gold-Based Nanomaterials

Yu-Shan Chen, Zhi-Wen Chen, Yu-Wen Yuan, Kuan-Chung Chen, and Ching-Ping Liu*

ACCESS

ABSTRACT: Although the regulation of the enzyme-like activities of nanozymes has stimulated great interest recently, the exploration of modulators makes it possible to enhance the catalytic performance of nanozymes, though doing so remains a big challenge. Herein, we systemically studied the effects of fluorescence quenchers on the peroxidase-like activity of bovine serum albumin-stabilized gold nanoclusters (BSA-AuNCs) based on photoinduced electron transfer (PET). We found that PET quenchers can not only quench the fluorescence of BSA-AuNCs but also regulate their intrinsic peroxidase-like activity. Importantly, both BSA and human serum albumin (HSA) could enhance the peroxidase-like activity of Cu²⁺, which provided a new sensing platform for distinguishing BSA and HSA from other thiol-containing biomolecules. The PET quenchers could also manipulate the peroxidase-like activity of polyvinylpyrrolidone-stabilized gold nanoparticles (PVP-AuNPs), which exhibited some opposite results between PVP-AuNPs and BSA-AuNCs. The opposite effects on BSA-AuNCs and PVP-AuNPs were speculated to highly depend on their surface properties. Our findings offer an efficient strategy for tuning the peroxidase-like activities of gold-based nanozymes.

INTRODUCTION

Nanomaterials capable of functions similar to those of natural enzymes (i.e., nanozymes) have stimulated much research interest because of their striking features, such as low cost and robustness. Currently, nanozymes are known to have various enzyme-like characteristics, such as those of peroxidase, oxidase, glucose oxidase, catalase, and superoxide dismutase. Typically, when nanozymes can catalyze hydrogen peroxidase (H₂O₂) oxidizing substrates and yielding products similar to those observed in peroxidase-catalyzed reactions, such nanozymes possess the peroxidase-like activity. Nanozymes with such activity have been widely used in the fields of biosensing and immunoassay, leading to the formation of a large family. On the basis of the catalytic cycle of horseradish peroxidase (HRP), the mechanism for nanozymes has been proposed to involve two major processes. One is the electron transfer processes among nanozymes, H₂O₂, and peroxidase substrates; the other is the formation of hydroxyl radicals (•OH), which act as the key intermediate during the peroxidase mimic reactions. However, the exact mechanisms of nanozymes may depend on the natures of different nanomaterials.

Recently, fluorescent metal nanoclusters with an ultrasmall size (<2 nm) have exhibited great promise as optical reporters in fluorescence-based sensing techniques for elucidating photophysical science, and thus they hold potential as research tools. In addition to their fluorescence, the catalytic properties of metal nanoclusters are also attractive because of their size-specific electronic and geometric structures. Among the various metal nanoclusters, bovine serum albumin-stabilized gold nanoclusters (BSA-AuNCs) were first reported to possess excellent peroxidase-like activity because of their small size, high surface-to-volume ratio, and strong affinity to the peroxidase substrate 3,3′,5,5′-tetramethylbenzidine (TMB). Subsequently, a dual fluorescence and colorimetric sensor was developed because dopamine (DA) can not only quench the fluorescence of BSA-AuNCs via photo-induced electron transfer (PET) but also inhibit their intrinsic peroxidase-like activity. That work has led to the intriguing issue of whether fluorescence quenchers may affect the peroxidase-like activity of BSA-AuNCs. To date, the fluorescence-quenching mechanisms of BSA-AuNCs have been widely studied and can be separated into two categories: one involving the aggregation and etching of the main Au component and the other relying on the PET and fluorescence resonance energy transfer (FRET) between BSA-AuNCs and quenchers. It is straightforward that aggregation and etching of BSA-AuNCs inevitably diminish their peroxidase-like activity because of
direct loss of surface accessibility (i.e., active sites). Comparatively, the influences of PET and FRET quenchers on the peroxidase-like activity of BSA-AuNCs are obscure because no chemical interaction takes place between such quenchers and BSA-AuNCs without irradiation. PET pairs can facilitate electron transfer from the donor to the acceptor based on their redox potentials, and then the energy in the excited states is dissipated nonradiatively. The efficiency of the PET process is mainly controlled by the spatial distance between the donor and the acceptor, the relationship between excitation and transfer processes, the free energy of the reaction, and the polarity of solvents. It remains unclear whether such catalytic properties of fluorescent nanozymes can be manipulated by their corresponding fluorescence quenchers. Accordingly, BSA-AuNCs are appropriate for investigating the effects of PET quenchers on their peroxidase-like activity.

In this work, we systemically studied the effects of PET quenchers on the peroxidase-like activity of BSA-AuNCs. Six quenchers including Hg^{2+}, Cu^{2+}, DA, ascorbic acid (AA), 3,3′-diethyliatricarbocyanine iodide (DTTC I), and nitrite ions (i.e., NO_2^−) were chosen because all of them could quench the fluorescence of BSA-AuNCs via the PET process. The possible mechanism to manipulate the peroxidase-like activity of BSA-AuNCs by PET quenchers was explored and discussed. In addition, the PET quenchers were also applied to regulate the peroxidase-like activity of AuNPs. Our findings offer an efficient strategy for tuning the peroxidase-like activities of gold-based nanozymes and even make it possible to enhance catalytic performance, which remains a big challenge.

## RESULTS AND DISCUSSION

### Peroxidase-like Activity of BSA-AuNCs with Fluorescence Quenchers.

The BSA-AuNCs were prepared using BSA as a template and reducing agent according to a previous report. The transmission electron microscopy (TEM) images, UV−vis absorption, and fluorescence spectra of BSA-AuNCs were obtained as shown in Figure S1, and all were consistent with the previous report. Figure 1A shows that these PET quenchers could quench the fluorescence of BSA-AuNCs under UV light (at 254 nm) irradiation. Subsequently, the peroxidase-like activities of BSA-AuNCs in the absence and presence of these quenchers were determined based on the oxidation of the peroxidase substrate (i.e., TMB) to generate the blue product (i.e., the oxidized TMB). Because BSA-AuNCs possessed the intrinsic peroxidase-like activity, the colorless TMB turned blue in the H_2O_2/TMB system. Interestingly, the additions of Hg^{2+}, DA, and AA could inhibit the peroxidase-like activity of BSA-AuNCs, whereas Cu^{2+}, DTTC I, and NO_2^− could not. In other words, fluorescence quenching did not necessarily result in the inhibition of the peroxidase-like activity of BSA-AuNCs. Figure 1B shows the behaviors of these PET quenchers alone in the H_2O_2/TMB system. Among them, Cu^{2+}, DTTC I, and NO_2^− could cause oxidation of TMB to produce a blue color with the major absorbance around 652 nm (i.e., the oxidized TMB). In addition, only NO_2^− could directly interact with TMB in the absence of H_2O_2 (Figure 1C). It should be noted that DTTC I exhibited a weak absorption feature over 600−750 nm, which was distinguishable from the oxidized TMB with the absorption band around ~650 nm. Cu^{2+} has been reported to possess the peroxidase-like activity, which was consistent with our observation (Figure 1B). As for DTTC I, its influence on the peroxidase-like activity of BSA-AuNCs will be discussed below. The quencher NO_2^− was excluded from the following experiments because of the interference of the peroxidase-like activity. These observations indicated that PET quenchers can...
not only quench the fluorescence of BSA-AuNCs but also regulate their intrinsic peroxidase-like activity.

The peroxidase-like activities of BSA-AuNCs inhibited by DA, Hg²⁺, and AA (Figure 1A) were the same as reported previously.⁹,²¹,²² The inhibition mechanism of Hg²⁺ has been ascribed to the active site of Au⁺ on the surface of BSA-AuNCs being blocked by Hg²⁺ through the formation of metallophilic bonding.²¹ The peroxidase-like activity stems from the ability to decompose H₂O₂ into •OH, which was the key intermediate for the oxidation of peroxidase substrates.⁶ AA and DA are both antioxidants,²³,²⁴ which can efficiently react with reactive oxygen species such as •OH and H₂O₂ to prevent the oxidation of peroxidase substrates, thereby diminishing the peroxidase-like activity of BSA-AuNCs. Therefore, if the PET quenchers were antioxidants or interacted with active sites,
peroxidase-like activity of BSA-AuNCs could be inhibited effectively.

Enhancement of the Peroxidase-like Activity of BSA-AuNCs. Although the additions of Cu$^{2+}$ and DTTC I quenched the fluorescence of BSA-AuNCs, they did not inhibit the corresponding peroxidase-like activity (Figure 1A). In contrast, an apparent increase in absorbance at 652 nm was observed with greater additions of Cu$^{2+}$ into the BSA-AuNCs solution (Figure 2A), indicating that the peroxidase-like activity was enhanced by Cu$^{2+}$. Previous work revealed that Cu$^{2+}$ could bind to BSA, leading to fluorescence quenching of BSA-AuNCs through electron transfer. It was speculated that Cu$^{2+}$ preferentially interacted with the outside BSA rather than with the interior AuNCs; if so, the peroxidase-like activity could be attributed to Cu$^{2+}$ and BSA-AuNCs simultaneously, leading to an increase in the catalytic performance of BSA-AuNCs. Another notable feature was that the mixture of BSA and Cu$^{2+}$ significantly enhanced the peroxidase-like activity of Cu$^{2+}$ by more than 50%, although BSA did not exhibit the peroxidase-like activity (Figure 2B). In addition, the enhancement factor was equivalent when human serum albumin (HSA) was used to replace BSA because HSA is structurally similar to BSA and Cu$^{2+}$ also has a strong affinity with HSA. Moreover, the similar affinities between Ni$^{2+}$ and either BSA or HSA led to no enhancement of the catalytic activity because Ni$^{2+}$ itself did not behave as a peroxidase mimic. Figure 2C displayed that the addition of BSA and HSA could both enhance the peroxidase-like activity of Cu$^{2+}$ significantly, but other protein thiols such as trypsin and lysozyme could not. Cu$^{2+}$ was recognized to have a high affinity with histidine because histidine typically exists in the protein chain. The surface functionalization of histidine on Fe$_3$O$_4$ nanoparticles has also been reported to mimic the active site of natural peroxidase enzyme (i.e., HRP), thereby enhancing the peroxidase-like activity of Fe$_3$O$_4$. However, the addition of histidine into Cu$^{2+}$ only slightly increased the peroxidase-like activity of Cu$^{2+}$ (Figure S2B), indicating that the interaction of Cu$^{2+}$ with histidine did not play the key role. It was speculated that other amino acids present in BSA or the structural coordination between Cu$^{2+}$ and BSA (or HSA) might affect the peroxidase-like activity of Cu$^{2+}$. These results indicated that such unexpected promotion of the catalytic activity of Cu$^{2+}$ provided the specificity of the sensing platform for distinguishing either BSA or HSA (Figure S3) from other biological thiols.

Figure 2D shows that the peroxidase-like activity of BSA-AuNCs was increased by adding DTTC I. DTTC I is a quaternary ammonium salt and thus can dissociate I$^-$ ions in aqueous solution. I$^-$ ions have been reported to catalyze the oxidation of TMB in the presence of H$_2$O$_2$ and DTTC I was also observed to catalyze the TMB/H$_2$O$_2$ system (see Figure 1C). Accordingly, the enhancement mechanism was considered to be highly associated with I$^-$ ions from DTTC I. To verify this possibility, DTTC I was replaced with NaI and KI as the source of I$^-$ ions. As shown in Figure 2E,F, the peroxidase-like activity of BSA-AuNCs was significantly increased when BSA-AuNCs were premixed with either NaI or KI in the H$_2$O$_2$/TMB system. In addition, the fluorescence of BSA-AuNCs could not be quenched by I$^-$ ions when adding either NaI or KI into the solution of BSA-AuNCs (Figure S4). Therefore, the enhancement of the peroxidase-like activity of BSA-AuNCs by DTTC I resulted from the corresponding counter ions of I$^-$ in DTTC I. This finding indicated that the chemical reaction between I$^-$ ions and TMB was much more dominant than the PET relation between BSA-AuNCs and the cyanine dyes.

Peroxidase-like Activity of BSA-AuNCs Mediated by Light Irradiation. The PET process takes place upon irradiation, which may contribute to the peroxidase-like activity of BSA-AuNCs in the presence of their PET quenchers. It should be noted that the fluorescence quenching of BSA-AuNCs by PET quenchers was typically achieved at neutral pH under UV light excitation (at 254 or 365 nm). However, the peroxidase-like activity should be examined under an acidic condition (pH 4), and H$_2$O$_2$ was easily photolyzed by UV light to produce *OH, resulting in the inference of the peroxidase-like activity. In addition, DTTC I and NO$_2^-$ should be excluded from the experiments upon irradiation because they can oxidize TMB in the presence of H$_2$O$_2$ (Figure 1B). For this purpose, the peroxidase-like activity of BSA-AuNCs with PET quenchers under an acidic condition (pH 4) was investigated without and with visible light irradiation (as shown in Figure S5). Figure 3A shows that the peroxidase-like activity of BSA-AuNCs was significantly enhanced upon visible light irradiation, which might be attributable to the oxidase-like activity of BSA-AuNCs by visible light activation. However,
the addition of Hg\(^{2+}\), Cu\(^{2+}\), DA, and AA did not benefit the peroxidase-like activity of BSA-AuNCs during visible light irradiation (Figure 3B). Similarly, Hg\(^{2+}\), DA, and AA still predominantly inhibited the catalytic performance of BSA-AuNCs, and Cu\(^{2+}\) seemed harmful to the effect of irradiation. It should be noted that the fluorescence quenching of BSA-AuNCs by Cu\(^{2+}\) could still take place at pH 4 (as shown in Figure S6). A previous work reported that hot electrons generated by irradiating laser (at 532 nm) were recognized as the key factor in the enhancement of the peroxidase-like activity of AuNPs.\(^{32}\) It was speculated that hot electrons might preferentially transfer to PET quenchers such as Cu\(^{2+}\), thereby reducing the enhancement effect of irradiation on the peroxidase-like activity of BSA-AuNCs.

**Extension from AuNCs to AuNPs.** Because these PET quenchers could manipulate the peroxidase-like activity of BSA-AuNCs, it was proposed that they might function similarly when gold nanoparticles (AuNPs) were used instead of BSA-AuNCs. To investigate this possibility, polyvinylpyrrolidone-stabilized AuNPs (PVP-AuNPs) were prepared\(^ {33}\) as shown in Figure S7 because of their high stability\(^ {34}\) and intrinsic peroxidase-like activity.\(^ {35}\) Figure 4 presents the peroxidase-like activities of PVP-AuNPs in the absence and presence of these PET quenchers as stated above. The catalytic activity of PVP-AuNPs was retained by the addition of Hg\(^{2+}\) and Cu\(^{2+}\) but inhibited by DA, AA, and DTTC I. Interestingly, the effects of Hg\(^{2+}\) and DTTC I on PVP-AuNPs were completely opposite to those on BSA-AuNCs. Because the PVP-AuNPs (∼10 nm) were relatively large and easy to separate from quenchers by centrifugation, whether the regulation of the catalytic activity by quenchers was reversible could be examined. More significantly, the peroxidase-like activity of PVP-AuNPs was almost recovered after centrifugation to remove AA, Hg\(^{2+}\), and Cu\(^{2+}\), respectively, indicating that no chemical reactions occurred between the AuNPs and quenchers. Unlike BSA-AuNCs, Hg\(^{2+}\) had no influence on the peroxidase-like activity of PVP-AuNPs, similar to citrate-stabilized AuNPs in the presence of Hg\(^{2+}\).\(^ {36}\) Because Hg\(^{2+}\) could be reduced to Hg\(^{0}\) by either citrate ions or the reducing agent NaBH\(_4\), it was considered that PVP-AuNPs might adsorb BH\(_4^-\) on the surface of AuNPs\(^ {37}\) and then Hg\(^{2+}\) was preferentially reduced to Hg\(^{0}\). However, in the preparation of BSA-AuNCs, BSA was typically used to replace NaBH\(_4\) as the reducing agent. Accordingly, Hg\(^{2+}\) might favor blocking the active site of Au\(^+\) on BSA-AuNCs rather than being reduced to Hg\(^{0}\). To further confirm this, X-ray photoelectron spectroscopy (XPS) was used to verify the change of Au\(^+\) for PVP-AuNPs and BSA-AuNCs in the absence and presence of Hg\(^{2+}\), respectively. Figure S6 shows the XPS spectra of Au 4f\(_{7/2}\) and 4f\(_{5/2}\), respectively. Each band could be deconvoluted into two distinct components of Au\(^{+}\) and Au\(^0\), and the percentage of surface Au\(^+\) was thereby calculated based on the peak areas of Au\(^{+}\) and Au\(^0\). Obviously, the addition of Hg\(^{2+}\) did not change the percentage of Au\(^+\) on PVP-AuNPs (Figure S8A), whereas it did change that on BSA-AuNCs (Figure S8B). Moreover, the consumption of Hg\(^{2+}\) by NaBH\(_4\) could retard the inhibition of the peroxidase-like activity of BSA-AuNCs (Figure S9). Taken together, although the detailed mechanism of PVP-AuNPs against Hg\(^{2+}\) blocking active sites was still unclear, these results indicated that whether Hg\(^{2+}\) is reduced or not could regulate the peroxidase-like activity of AuNPs and AuNCs.

In addition to Hg\(^{2+}\), the effect of DTTC I on PVP-AuNPs also exhibited the opposite result from BSA-AuNCs. According to the literature, I\(^-\) ions inhibited the peroxidase-like activity of casein-stabilized AuNPs because of the formation of the Au–I bond.\(^ {39}\) Similarly, the peroxidase-like activity of PVP-AuNPs could be inhibited by I\(^-\) ions from DTTC I, KI, and NaI, respectively (Figure S10). In addition, the removal of DTTC I did not lead to the recovery of the catalytic activity of PVP-AuNPs (Figure 4). These observations indicated that DTTC I decreased the peroxidase-like activity of PVP-AuNPs because I\(^-\) ions blocked active sites via the formation of the Au–I bond.\(^ {39}\) The next question was why I\(^-\) ions could not diminish the peroxidase-like activity of BSA-AuNCs. Because I\(^-\) ions could not penetrate the interior of the BSA matrix,\(^ {30,42}\) it was speculated that free I\(^-\) ions predominantly reacted with TMB and H\(_2\)O\(_2\), rather than blocking active sites on BSA-AuNCs. Therefore, the opposite effects of Hg\(^{2+}\) and DTTC I on BSA-AuNCs and PVP-AuNPs further demonstrated that the peroxidase-like activities of AuNPs and AuNCs were highly dependent on their surface properties. Our findings could offer efficient strategies for tuning the peroxidase-like activities of gold-based nanomaterials.

## CONCLUSIONS

In this work, we systemically studied the effects of PET quenchers on the peroxidase-like activity of BSA-AuNCs. Our results showed that fluorescence quenching of BSA-AuNCs by their PET quenchers did not necessarily correspond to the inhibition of their peroxidase-like activity. Impressively, the
peroxidase-like activity of BSA-AuNCs was increased by Cu²⁺ and DTTC I, respectively. Because of the specificity of Cu²⁺ binding to BSA rather than to AuNCs, the combination of Cu²⁺ and BSA-AuNCs could act as two sources of peroxidase mimics. Unexpectedly, both BSA and HSA could enhance the peroxidase-like activity of Cu²⁺, which provided a good sensing platform for distinguishing BSA and HSA from other biothiols. As for DTTC I, enhancement of the peroxidase-like activity of BSA-AuNCs resulted from the corresponding counter ions of I⁻ rather than from the cyanine dye. In addition, the peroxidase-like activity of BSA-AuNCs with PET quenchers was also investigated without and with visible light irradiation, but the presence of PET quenchers decreased the enhancement effect of irradiation on the catalytic activity. It was speculated that hot electrons might preferentially transfer to PET quenchers such as Cu²⁺, thereby reducing the enhancement effect of irradiation on the peroxidase-like activity of BSA-AuNCs. Moreover, the PET quenchers could also manipulate the peroxidase-like activity of PVP-AuNPs, which exhibited some opposite results between PVP-AuNPs and BSA-AuNCs. The opposite effects of Hg²⁺ and DTTC I on BSA-AuNCs and PVP-AuNPs further demonstrated that the peroxidase-like activities of AuNPs and AuNCs were highly dependent on their surface properties. Our findings offer an efficient strategy for tuning the peroxidase-like activity of gold-based nanozymes and even make it possible to enhance catalytic performance, which typically is relatively difficult to achieve.

### EXPERIMENTAL SECTION

#### Chemicals and Reagents.

BSA, HSA, hydrogen tetrachloroaurate(III) tetrahydrate (HAuCl₄·3H₂O), sodium hydroxide (NaOH), TMB, DA, AA, mercury(II) chloride (HgCl₂), copper(II) chloride (CuCl₂), sodium nitrite (NaNO₂), nickel(II) nitrate hexahydrate (Ni(NO₃)₂·6H₂O), and DTTC I were all purchased from Sigma. All reagents were used without further purification.

#### Preparation of BSA-AuNCs.

BSA-AuNCs were prepared according to a previous report with minor modification. Briefly, the aqueous HAuCl₄ solution (5 mL, 10 mM) was added into the BSA solution (5 mL, 50 mg mL⁻¹) in a 37 °C water bath with stirring for 30 min. Subsequently, the NaOH (0.5 mL, 1.0 M) solution was added to the mixture under 37 °C incubation with vigorous stirring. The color of the mixture changed from pale yellow to deep brown, and the resulting solution was continuously stirred for 12 h. BSA-AuNCs were obtained after centrifugation at 6000 rpm to remove excess reactants and washed with DI water at least three times. The final solutions were stored at 4 °C in a refrigerator for further experiments.

#### Preparation of PVP-AuNPs.

The preparation of PVP-AuNPs followed a seed-mediated growth method that allows easy control of the size of AuNPs. All glassware and stir bars were cleaned in aqua regia (1:3 HNO₃:HCl) and rinsed with DI water before use for the preparation of PVP-AuNPs. Initially, 555 mg of PVP (molecular weight 10 kDa) was added into an aqueous solution of HAuCl₄ (1 mM, 50 mL), and the mixture was stirred at 4 °C for 30 min. Subsequently, 5 mL NaBH₄ (100 mM) was rapidly added into the mixture under vigorous stirring. After stirring at 4 °C for another 30 min, the resulting solution was kept as seeds for preparation of PVP-AuNPs. Next, 1 mL HAuCl₄ (50 mM) was diluted by DI water (35 mL), 555 mg PVP was added, and the solution was stirred at 4 °C for 30 min. The above solution was added to 10 mL of the seed solution, and the mixture was stirred for another 30 min. After that, 15 mL AA (5 mM) was slowly dropped into the above mixture and the solution was stirred at 4 °C for 2 h. Finally, PVP-AuNPs were obtained after centrifugation at 10,000 rpm to remove excess reactants and washed with DI water at least three times. The solution of PVP-AuNP was stored at 4 °C in a refrigerator for further experiments.

#### Characterization.

UV–vis absorption spectra were obtained using a JASCO V-670 spectrophotometer. Emission spectra were recorded using a JASCO FP-8300 fluorescence spectrophotometer. TEM images were taken with a JEM 2100 FE (JEOL) transmission electron microscope at an accelerating voltage of 200 kV. High resolution XPS (ULVAC-PHI XPS, PHI Quantera SXM) was used to determine atomic compositions.

#### Examination of the Peroxidase-like Activity of BSA-AuNCs.

The peroxidase-like activity of BSA-AuNCs was evaluated in acetate buffer (pH 4, 0.1 M) as the peroxidase substrate in the presence of 50 mM H₂O₂. HRP was used as the positive control. For the added fluorescence quenchers, various concentrations of quenchers, including DA, AA, Hg²⁺, Cu²⁺, DTTC I, and NO₃⁻, were first mixed with BSA-AuNCs (10 μL, 0.5 mM) for 10 min. Subsequently, acetate buffer, H₂O₂, and TMB were added. After the solution was incubated for 30 min, the color change of the resulting solution was observed with the UV–vis absorption spectrophotometer and photographs were taken. To monitor the catalytic oxidation of TMB with time, absorption values were collected at 652 nm for TMB using the scanning kinetic mode of the UV–vis spectrophotometer.

#### Detection of the Peroxidase-like Activity of BSA-AuNCs under Light Irradiation.

To examine the peroxidase-like activity of BSA-AuNCs enhanced under light irradiation, experiments were carried out using 5 μM BSA-AuNCs solution in a reaction volume of 1 mL acetate buffer (pH 4, 0.1 M) with 0.2 mM TMB and 50 mM H₂O₂. The above solution was irradiated with a 300 W Xe lamp equipped with a cutoff filter (λ ≥ 400 nm) to provide visible light.

#### ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c02956.

Absorption and emission spectra and TEM images for characterization of BSA-AuNCs; absorption spectra for the peroxidase-like activity of Cu²⁺ in the presence of Histidine; peroxidase-like activity of Cu²⁺ with HSA and the possibility of application as a sensing platform; examination of the fluorescence quenching of BSA-AuNCs by I⁻ ions; effect of visible light irradiation on the peroxidase-like activity of BSA-AuNCs with quenchers; fluorescence quenching of BSA-AuNCs by Cu²⁺ at pH 4; TEM images and the size distribution of PVP-AuNPs; deconvoluted XPS spectra of PVP-AuNPs and BSA-AuNCs without and with the addition of Hg²⁺; effect of NaBH₄ on the peroxidase-like activity of BSA-AuNCs; and peroxidase-like activities of PVP-AuNPs in the presence of DTTC I, KI, and NaI, respectively (PDF).
AUTHOR INFORMATION

Corresponding Author
Ching-Ping Liu — Department of Chemistry, Fu Jen Catholic University, New Taipei City 242062, Taiwan; orcid.org/0000-0003-3510-8583; Email: 129723@mail.fju.edu.tw

Authors
Yu-Shan Chen — Department of Chemistry, Fu Jen Catholic University, New Taipei City 242062, Taiwan
Zhi-Wen Chen — Department of Chemistry, Fu Jen Catholic University, New Taipei City 242062, Taiwan
Yu-Wen Yuan — Department of Chemistry, Fu Jen Catholic University, New Taipei City 242062, Taiwan
Kuan-Chung Chen — Department of Chemistry, Fu Jen Catholic University, New Taipei City 242062, Taiwan

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.0c02956

Author Contributions
The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Notes
The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank the Ministry of Science and Technology of Taiwan (MOST-108-2113-M-030-009) for providing financial support for this research. Special thanks go to Swee Lan Cheah for her assistance on XPS measurements.

REFERENCES

(1) Wu, J.; Wang, X.; Wang, Q.; Lou, Z.; Li, S.; Zhu, Y.; Qin, L.; Wei, H. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). Chem. Soc. Rev. 2019, 48, 1004–1076.
(2) Wei, H.; Wang, E. Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes. Chem. Soc. Rev. 2013, 42, 6060–6093.
(3) Wang, Q.; Wei, H.; Zhang, Z.; Wang, E.; Dong, S. Nanozyme: An emerging alternative to natural enzyme for biosensing and immunosassay. TrAC, Trends Anal. Chem. 2018, 105, 218–224.
(4) Rodríguez-López, J. N.; Lowe, D. J.; Hernández-Ruiz, J.; Hiner, A. N. P.; García-Cánovas, F.; Thorneley, R. N. F. Mechanism of Reaction of Hydrogen Peroxide with Horseradish Peroxidase: Identification of Intermediate in the Catalytic Cycle. J. Am. Chem. Soc. 2001, 123, 11838–11847.
(5) Mu, J.; Wang, Y.; Zhao, M.; Zhang, L. Intrinsic peroxidase-like activity and catalase-like activity of Co3O4 nanoparticles. Chem. Commun. 2012, 48, 2540–2542.
(6) Chen, Z.; Yin, J.-J.; Zhou, Y.-T.; Zhang, Y.; Song, L.; Song, M.; Hu, S.; Gu, N. Dual enzyme-like activities of iron oxide nanoparticles and their implication for diminishing cytotoxicity. ACS Nano 2012, 6, 4001–4012.
(7) Yamazoe, S.; Koyasu, K.; Tsukida, T. Nonscalable oxidative catalysis of gold clusters. Acc. Chem. Res. 2014, 47, 816–824.
(8) Wang, X.-X.; Wu, Q.; Shan, Z.; Huang, Q.-M. BSA-stabilized Au clusters as peroxidase mimetics for use in xanthine detection. Biosens. Bioelectron. 2011, 26, 3614–3619.
(9) Tao, Y.; Lin, Y.; Ren, J.; Qu, X. A dual fluorometric and colorimetric sensor for dopamine based on BSA-stabilized Au nanoclusters. Biosens. Bioelectron. 2013, 42, 41–46.
(10) Li, H.; Zhu, W.; Wan, A.; Liu, L. The mechanism and application of the protein-stabilized gold nanocluster sensing system. Analyst 2017, 142, 567–581.
(11) Hu, L.; Han, S.; Parveen, S.; Yuan, Y.; Zhang, L.; Xu, G. Highly sensitive fluorescent detection of trypsin based on BSA-stabilized gold nanoclusters. Biosens. Bioelectron. 2012, 32, 297–299.
(12) Liu, Y.; Ai, K.; Cheng, X.; Huo, L.; Lu, L. Gold-nanocluster-based fluorescent sensors for highly sensitive and selective detection of cyanide in water. Adv. Funct. Mater. 2010, 20, 951–956.
(13) Hu, D.; Sheng, Z.; Gong, P.; Zhang, P.; Cai, L. Highly selective fluorescent sensor for Hg2+ based on bovine serum albumin-capped gold nanoclusters. Analyst 2010, 135, 1411–1416.
(14) Zhou, W.; Cao, Y.; Sui, D.; Guan, W.; Lu, C.; Xie, J. Ultrastable BSA-capped gold nanoclusters with a polymer-like shielding layer against reactive oxygen species in living cells. Nanoscale 2016, 8, 9614–9620.
(15) Marcus, R. A.; Sutin, N. Electron transfers in chemistry and biology. Biochim. Biophys. Acta, Rev. Bioenerg. 1985, 811, 265–322.
(16) Gray, H. B.; Winkler, J. R. Electron transfer in proteins. Annu. Rev. Biochem. 1996, 65, 537–561.
(17) Rehm, D.; Wellner, A. Kinetics of fluorescence quenching by electron and H-atoms transfer. Isr. J. Chem. 1970, 8, 259–271.
(18) Xie, J.; Zheng, Y.; Ying, J. Y. Protein-directed synthesis of highly fluorescent gold nanoclusters. J. Am. Chem. Soc. 2009, 131, 881–889.
(19) Wang, G.-L.; Jin, L.-Y.; Dong, Y.-M.; Wu, X.-M.; Li, Z.-J. Intrinsic enzyme mimicking activity of gold nanoclusters upon visible light triggering and its application for colorimetric tspyn detection. Biosens. Bioelectron. 2015, 64, 523–529.
(20) Zheng, A.; Zhang, X.; Gao, J.; Liu, X.; Liu, J. Peroxidase-like catalytic activity of copper ions and its application for highly sensitive detection of glycypic A. Anal. Chem. Acta 2016, 941, 87–93.
(21) Zhu, R.; Zhou, Y.; Wang, X.-L.; Liang, L.-P.; Long, Y.-J.; Wang, Q.-L.; Zhang, H.-J.; Huang, X.-X.; Zheng, H.-Z. Detection of H2g based on the selective inhibition of peroxidase-like activity of BSA-Au clusters. Talanta 2013, 117, 127–132.
(22) Wang, X.; Wu, P.; Hou, X.; Lv, Y. An ascorbic acid sensor based on protein-modified Au nanoclusters. Analyst 2013, 138, 229–233.
(23) Buettner, G. R. The pecking order of free radicals and antioxidants: lipid peroxidation, alpha-tocopherol, and ascorbate. Arch. Biochem. Biophys. 1993, 300, 535–543.
(24) Yen, G.-C.; Hsieh, C.-L. Antioxidant Effects of Dopamine and Related Compounds. Bisci., Biotehiol., Biochem. 1997, 61, 1646–1649.
(25) Durgadas, C. V.; Sharma, C. P.; Sreenivasan, K. Fluorescent gold clusters as nanosensors for copper ions in live cells. Analyst 2011, 136, 933–940.
(26) Carter, D. C.; Ho, J. X. Structure of serum albumin. Adv. Protein Chem. 1994, 45, 153–202.
(27) Selvaprakash, K.; Chen, Y.-C. Using protein-encapsulated gold nanoclusters as photoluminescent sensing probes for biomolecules. Biosens. Bioelectron. 2014, 61, 88–94.
(28) Fan, K.; Wang, H.; Xi, J.; Liu, Q.; Meng, X.; Duan, D.; Gao, L.; Yan, X. Optimization of Fe3O4 nanzyme activity via single amino acid modification mimicking an enzyme active site. Chem. Commun. 2017, 53, 424–427.
(29) Hu, B.; Zhao, Y.; Zhu, H. Z.; Yu, S. H. Selective chromogenic detection of thiol-containing biomolecules using carbonaceous nanospheres loaded with silver nanoparticles as carrier. ACS Nano 2011, 5, 3166–3171.
(30) Liu, C.-P.; Wu, T.-H.; Liu, C.-Y.; Lin, S.-Y. Live-cell imaging of biotools via thiol/disulde exchange to trigger the photoinduced electron transfer of gold-nanodot sensor. Anal. Chim. Acta 2014, 849, 57–63.
(31) Banerjee, C.; Kuchlyan, J.; Banik, D.; Kundu, N.; Roy, A.; Ghosh, S.; Sarkar, N. Interaction of gold nanoclusters with IR light emitting cyanine dyes: a systematic fluorescence quenching study. Phys. Chem. Chem. Phys. 2014, 16, 17272–17283.
(32) Wang, C.; Shi, Y.; Dan, Y.-Y.; Nie, X.-G.; Li, J.; Xia, X.-H. Enhanced peroxidase-like performance of gold nanoparticles by hot electrons. Chem.—Eur. J. 2017, 23, 6717–6723.
(33) Yang, X.-W.; Zhang, G.-R.; Li, Y.-X.; Xu, B.-Q. Size-control of monodispersing gold nanoparticles via stepwise seed-mediated growth. *Acta Phys.-Chim. Sin.* 2009, 25, 2565–2569.

(34) Kim, Y.; Smith, J. G.; Jain, P. K. Harvesting multiple electron–hole pairs generated through plasmonic excitation of Au nanoparticles. *Nat. Chem.* 2018, 10, 763–769.

(35) Liu, C.-P.; Chen, K.-C.; Su, C.-F.; Yu, P.-Y.; Lee, P.-W. Revealing the active site of gold nanoparticles for the peroxidase-like activity: the determination of surface accessibility. *Catalysts* 2019, 9, 517.

(36) Long, Y. J.; Li, Y. F.; Liu, Y.; Zheng, J. J.; Tang, J.; Huang, C. Z. Visual observation of the mercury-stimulated peroxidase mimetic activity of gold nanoparticles. *Chem. Commun.* 2011, 47, 11939–11941.

(37) Deraedt, C.; Salmon, L.; Gatard, S.; Ciganda, R.; Hernandez, R.; Ruiz, J.; Astruc, D. Sodium borohydride stabilizes very active gold nanoparticle catalysts. *Chem. Commun.* 2014, 50, 14194–14196.

(38) Park, J.-W.; Shumaker-Parry, J. S. Strong resistance of citrate anions on metal nanoparticles to desorption under thiol functionalization. *ACS Nano* 2015, 9, 1665–1682.

(39) Liu, Y.; Xiang, Y.; Zhen, Y.; Guo, R. Halide ion-induced switching of gold nanozyme activity based on Au–X interactions. *Langmuir* 2017, 33, 6372–6381.

(40) Lehrer, S. Solute perturbation of protein fluorescence. Quenching of the tryptophyl fluorescence of model compounds and of lysozyme by iodide ion. *Biochemistry* 1971, 10, 3254–3263.

(41) Maruthamuthu, M.; Selvakumar, G. Selective quenching of tryptophanyl fluorescence in bovine serum albumin by the iodide ion. *Proc. Indian Acad. Sci., Chem. Sci.* 1995, 107, 79–86.