Aggregation-Enhanced Emission of Gold Nanoclusters Induced by Serum Albumin and Its Application to Protein Detection and Fabrication of Molecular Logic Gates

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Supporting Information

ABSTRACT: Exploring aggregation-enhanced emission (AEE) of gold nanoclusters (Au NCs) is beneficial for extending their applications in sensing and molecular information processing. Herein, we present the first report of a protein-induced AEE effect of Au NCs. When human serum albumin (HSA) is mixed with glutathione-capped Au NCs under appropriate pH conditions, the Au NCs undergo extensive aggregation and exhibit significantly enhanced emission, attributed to the electrostatic and hydrophobic interactions between HSA and the NCs. Such an AEE effect is specific to serum albumin over a variety of other proteins, which facilitates the development of a facile approach for HSA determination. This sensing method displays satisfactory recoveries of 96.0–98.7% when it is applied to HSA detection in artificial urine. Moreover, the AEE effect is suited to the fabrication of AND and INHIBIT logic gates by using HSA and pH/protein-binding drug as inputs and the emission as output.

1. INTRODUCTION

Ligand-capped luminescent gold nanoclusters (Au NCs) have attracted extensive attention for facile syntheses, high photostability, and good biocompatibility. Particularly, the luminescence of Au NCs can readily be modiﬁed using various analytes, including metal cations, inorganic anions, and biomolecules, which provide a basis for developing sensing methods for chemical/biological detection.1−3 In most of the cases, interactions between the Au NCs and analytes cause luminescence quenching of the NCs. For example, several Hg2+−ion-sensing methods have been designed by using the effective luminescence quenching of Au NCs through metallophilic Hg2+−Au+ interactions.4−6 Chemical oxidation also causes the luminescence quenching of Au NCs, which can be used for sensing CN− and H2O2.7,8 Recently, a limited but increasing number of studies have focused on the luminescence enhancement of Au NCs for sensing application. For example, Banerjee et al. report an As3+ ion assay based on the luminescence enhancement of dicysteine-capped Au NCs through the formation of a charge-transfer complex.9 Liu et al. develop a strategy for cysteine determination based on the enhanced emission of protein-capped Au NCs induced by attachment of cysteine on the NC surfaces.10

Aggregation-enhanced emission (AEE) is an intriguing optical phenomenon in which weakly luminescent species display remarkably enhanced emission in their aggregated states.11,12 While AEE is usually observed for organic molecules, it was demonstrated very recently that Au NCs also exhibit the AEE effect. For example, Pei et al. reported the AEE of Au NCs induced by Pb2+ ions,13 while Zhou et al. reported a similar phenomenon induced by Ag+ ions.14 Yahia-Ammar et al. demonstrated the AEE effect of Au NCs resulting from electrostatic interactions with cationic polymers, such as poly(allylamine hydrochloride) and polyethylenimine.15

Human serum albumin (HSA) is a vital protein for human beings since it regulates intravascular osmotic pressure and transports bioactive molecules such as vitamins, hormones, and drugs in bloodstream. For a healthy person, HSA is normally present in blood rather than in urine due to filtering by the kidneys. However, when the kidneys are damaged, high levels of HSA are presented in urine, which is referred to as “albuminuria” and is a key clinical indicator of diabetes, renal, and cardiovascular diseases.16 Herein, we reported the first study using protein, that is, HSA, to induce the aggregation of glutathione-capped Au NCs (GSH−Au NCs), which, in turn, signiﬁcantly enhanced the emission of the NCs. By taking advantage of the AEE effect, we developed a luminescence enhancement strategy for protein detection, which showed excellent sensitivity and selectivity for serum albumin over a variety of other proteins. Furthermore, molecular logic gates...
capable of performing AND and INHIBIT operations were fabricated based on the AEE effect.

2. RESULTS AND DISCUSSION

2.1. Aggregation-Enhanced Emission of GSH−Au NCs in the Presence of HSA. GSH−Au NCs were prepared following Xie’s method.20 The as-prepared Au NCs exhibited absorption/emission bands around 390/618 nm, respectively (Figure S1). A dispersion of GSH−Au NCs in phosphate buffer (pH 4.0) exhibited weak luminescence while being irradiated by ultraviolet (UV) light, which was significantly enhanced upon the addition of HSA (Figure 1A). The luminescence enhancement of the NCs by HSA was then studied by monitoring the dependence of the enhancement factor, \( I/I_0 \), with incubation time, where \( I \) and \( I_0 \) represent luminescent emission intensities of the NCs at 618 nm with and without HSA, respectively. Figure 1B reveals that the \( I/I_0 \) value increased rapidly and plateaued over 15 min following the addition of HSA, while the luminescence of the Au NC dispersion remained unchanged in the absence of HSA, which highlighted the vital role played by HSA in enhancing the emission of the NCs.

Dynamic light scattering (DLS) analysis indicates that the hydrodynamic diameter of the GSH−Au NC was ca. 4 nm.
which increased significantly to ca. 570 nm upon the addition of HSA (Figure 1C), implying that the Au NCs may undergo extensive aggregation in the presence of HSA. TEM study reveals the NCs were well dispersed and ∼1.1 nm in size, while large aggregates with sizes of ∼60.9 nm were obtained upon the addition of HSA (inset, Figure 1D). The larger hydrodynamic diameters derived from DLS measurements than those determined by TEM observations are related to the presence of hydration shells around the Au NCs and the aggregates in aqueous media. Moreover, the UV−vis absorption spectra displayed in Figure 1D reveal a noticeable increase in background scattering in the absorbance of the Au NC dispersion after the introduction of HSA. Correspondingly, the transparent dispersion of GSH−Au NCs became slightly turbid after the addition of HSA (Figure S2) attributed to the aggregation of the NCs, which accounted for the increased background scattering in the UV−vis absorption spectrum. Moreover, the characteristic surface plasmon resonance peak of gold nanoparticles ∼520 nm did not appear in the UV−vis absorption spectrum, which excluded the formation of large gold nanoparticles upon mixing the GSH−Au NCs with HSA. These results were consistent with the AEE of Au NCs induced by polymers and metal ions.16−18

2.2. Possible Mechanism for the HSA-Induced AEE of GSH−Au NCs. Electrostatic interactions are essential for driving the polymer/metal cation-induced aggregation of metal nanoclusters.17,18 In our case, ζ-potential measurements reveal that GSH−Au NCs were negatively charged at pH 3.0−9.0, while HSA is an acidic protein with an isoelectric point (pI) of 4.7 (Figure 2A). Figure 2B shows that both I/I₀ and the hydrodynamic diameters of the Au NCs following the addition of HSA were highly pH dependent. A maximum I/I₀ value of 3.3 was observed at pH 4.0, in which electrostatic attractions between the negatively charged Au NCs (∼−4.8 mV) and the positively charged HSA (4.3 mV) lead to the aggregation of the NCs, followed by the enhanced luminescence. When pH of the dispersion was set to be 3.0, at which the Au NCs were less charged, or to 5.0, at which the HSA was less charged, obvious decreases in I/I₀ were observed, owing to the limited electrostatic attraction. The AEE effect was negligible at pH value higher than 6.0 since both the NCs and HSA were negatively charged at the high pH. Hence, we deduced that electrostatic interactions between the GSH−Au NCs and the HSA played important roles in inducing the AEE effect.

The effect of other protein with pI similar to or higher than that of HSA, including ovalbumin (4.7), carbonic anhydrase (CA, 6.1), immunoglobulin G (IgG, 6.6), hemoglobin (Hb, 6.8), lipase (6.9), horseradish peroxidase (HRP, 7.2), trypsin (8.7), RNase A (9.2), lysozyme (Lys, 10.8) or bovine serum albumin (BSA, 4.7), on the enhancement factor was further evaluated to understand the AEE effect. After being added into aqueous dispersions of the GSH−Au NCs at pH 4.0, as shown in Figure 3A, only HSA and BSA, a highly structural and functional analogue of HSA, can lead to the significant emission enhancement of GSH−Au NCs, while other proteins only induced slight changes in the enhancement factor. The fact that other proteins, except serum albumin, failed to trigger the AEE of GSH−Au NC indicated that electrostatic interactions between protein and the NCs alone are insufficient to promote the AEE effect. In addition to electrostatic attraction, it is likely that there exist other driving forces to

Figure 3. (A) Histogram of enhancement factor, I/I₀ at 618 nm of the Au NC dispersion upon addition of ovalbumin, CA, IgG, Hb, lipase, HRP, trypsin, RNase A, Lys, BSA, and HSA. (B) Circular dichroism (CD) spectra of HSA with and without the Au NCs. (C) Luminescence decay and (D) X-ray photoelectron spectroscopy (XPS) profiles of the Au NCs with and without HSA. Concentrations of all the proteins were set to be 500 nM in these experiments.
contribute to the AEE effect, which appear to be specific for serum albumin.

Serum albumin is a typical protein with several hydrophobic pockets and can bind to many organic probes through hydrophobic interactions.\textsuperscript{21–27} Circular dichroism (CD) spectra measurement was performed to further investigate the interaction of HSA with the Au NCs. As shown in Figure 3B, HSA had two negative CD bands ~208 and 218 nm, corresponding to the \( \alpha \)-helical structure in protein.\textsuperscript{23} While after HSA was mixed with a dispersion of GSH–Au NCs at pH 4.0, the bands at 208 and 218 nm almost disappeared, while a positive band emerged at 200 nm and a negative band appeared around 230 nm, highlighting the substantial change in the secondary structure of the protein, which was caused by the interaction of HSA with the NCs. Contents of the secondary structures calculated from the CD spectrum of HSA were 50\% for \( \alpha \)-helix, 12\% for \( \beta \)-sheet, 13\% for \( \beta \)-turn, and 25\% for random coil. After being mixed with the NC dispersion, the content of \( \alpha \)-helix decreased dramatically to 9\%, while those of \( \beta \)-sheet, \( \beta \)-turn, and random coil increased to 37, 25, and 29\%, respectively. Such changes in secondary structure suggested that the Au NCs possibly bound HSA to 37, 25, and 29\%, respectively.\textsuperscript{29} The hydrophobic interaction of HSA with GSH–Au NCs was further illustrated by a control experiment, in which the enhanced Au NC emission induced by HSA was greatly suppressed in the presence of guanidine hydrochloride (GdnHCl), a denaturant agent that destroys the hydrophobic interaction between proteins and organic probes (Figure S3).\textsuperscript{22} Accordingly, the specific HSA-induced AEE effect of the Au NCs was possibly ascribable to the greater hydrophobicity of HSA compared to most of the other proteins.\textsuperscript{29}

Time-resolved luminescence and X-ray photoelectron spectra (XPS) were acquired in order to shed light on the AEE effect. Decay curve of the Au NCs was fitted by a triexponential function, with lifetimes of 0.35 \( \mu s \) (18.03\%), 1.14 \( \mu s \) (47.85\%), and 4.68 \( \mu s \) (34.12\%) (Figure 3C). Such microsecond-level lifetimes indicated that emission of GSH–Au NC came from ligand-to-metal charge transfer/ligand-to-metal–metal charge transfer, which resulted in radiative relaxation.\textsuperscript{20,30} The lifetimes of the Au NCs were extended to 0.47 \( \mu s \) (13.14\%), 2.17 \( \mu s \) (46.15\%), and 7.18 \( \mu s \) (40.71\%) in the presence of HSA. Such prolonged decay times indicated the suppressed ligand-related nonradiative relaxation in the excited state, which might contribute to the strikingly enhanced emission of the NCs induced by HSA.\textsuperscript{18,26} Moreover, Au 4f\(_{7/2}\) and 4f\(_{5/2}\) XPS peaks of the Au NCs centered at 88.1 and 84.3 eV (Figure 3D), which indicated the presence of Au(0) and Au(I) in the NCs. Upon the addition of HSA, these peaks shifted to 83.5 and 86.9 eV, suggesting transformation of a part of the Au(I) into Au(0), which might also contribute to the AEE effect.\textsuperscript{31}

2.3. Method for HSA Determination Based on the AEE Effect. Based on the HSA-induced AEE of the Au NCs, a luminescence method for quantitative determination of HSA was developed. Figure 4A reveals that emission intensities of the NCs increased with the increasing HSA concentration. Figure 4B shows that the emission enhancement factor, \( I/I_0 \), is almost linear with the HSA concentration from 10 to 500 nM. The detection limit was ~1.6 nM, estimated by the IUPAC method, which was comparable to those of other probes and bioactive small molecules, on emission intensities of the nanoclusters was then evaluated.\textsuperscript{12} The presence of Na\(^+\) ions (3.0 mM), K\(^+\) ions (3.0 mM), NH\(_4\)\(^+\) ions (3.0 mM), Ca\(^2+\) ions (3.0 mM), Mg\(^{2+}\) ions (3.0 mM), glucose (25 mM), creatinine (Cre, 0.5 mM), uric acid (20 mM), urea (0.2 M), cysteine (Cys, 500 nM), homocysteine (Hcy, 500 nM), glutathione (GSH, 500 nM), and oxidized glutathione (GSSH, 500 nM) had almost negligible effect on the emission intensities of the NCs. Consequently, this method should be capable of sensing HSA in practical samples, such as artificial urine. In a typical experiment, 100 \( \mu L \) of artificial urine was spiked with defined concentrations of HSA, and then the mixtures were analyzed by using the AEE-based assay as well as a classical bicinchoninic acid (BCA) method. The results obtained

![Figure 4](https://example.com/figure4.png)
from the concentration calibration curve (Figure S4) for the AEE-based assay were consistent with those acquired using the BCA method (Table S2). In addition, our method exhibited excellent recoveries (96.0−98.7%) and relative standard deviations (<5.0%), highlighting its potential use for HSA detection in real samples.

2.4. AEE-Based Design of Molecular Logic Gates. Logic gates that transform chemical/biological input into measurable output have attracted significant attention due to potential applications to information processing at the molecular level.32,33 Based on the AEE effect associated with the Au NCs induced by HSA, we designed a platform capable of performing molecular logic operations. To implement the logic function, AEE-related factors such as HSA and pH (H+, OH−) were employed as inputs. The absence and presence of the input were designated to be “0” and “1”, respectively. On the other hand, the weak luminescence associated with dispersed GSH–Au NCs was defined to be output 0, while the enhanced
emission associated with the Au NC-HSA aggregates was defined to be output 1, with a threshold $I/I_0$ value of 2.0. As shown in Figure 5, an AND gate that provides an output of 1 when both inputs are 1 was fabricated using the pH-dependent AEE effect. The Au NC dispersion at pH 7.0 was defined to be the initial state. The two inputs for the AND gate were set to be HSA and H+. The Au NCs exhibited poor luminescence without any input (output 0). The addition of a single input, either HSA or H+ ions, into the Au NC dispersion (pH 7.0) was unable to trigger the AEE effect (output 0). Only when both inputs, that is, HSA and H+, were added simultaneously was AEE observed (output 1), which supported an AND function that transforms biochemical information, that is, the presence of HSA and environmental pH change, into a detectable luminescence signal.

An INHIBIT gate, in which the output is 1 when only one input is 1 (and the other input is 0), was constructed next. As shown in Figure 6, the initial state of the INHIBIT gate was set to be the GSH−Au NC dispersion at pH 4.0. HSA was used as one input and OH− as the other. The GSH−Au NCs exhibited AEE with only HSA as the input, to give an output signal of 1. The addition of OH− alone resulted in negligible change of the weak luminesces of the GSH−Au NCs (output 0). However, when the gate was subjected to both inputs together, AEE was inhibited by the neutral pH condition caused by the addition of OH− (output 0); hence, this configuration corresponded to an INHIBIT logic gate. The OH− ions can be replaced by another input capable of suppressing the AEE effect when designing INHIBIT gates. For example, an AEE-based INHIBIT gate can also be designed using ketoprofen instead of OH− ions as an input. Ketoprofen is a nonsteroidal anti-inflammatory drug that can competitively bind to HSA, thereby preventing interactions between HSA and the NCs (Figures 6A and S5). Considering that HSA is a model protein in molecular biology, the AEE-based INHIBIT gate might be potentially useful for the screening of drugs and the evaluation of their physiological activities.

3. CONCLUSIONS

In summary, we demonstrate that HSA is effective to induce the aggregation-enhanced emission (AEE) of GSH−Au NCs. To the best of our knowledge, this is the first study of a protein-triggered AEE effect of the Au NCs. It is identified that both hydrophobic and electrostatic interactions between HSA and the Au NCs contribute to the AEE effect. Such an AEE effect involving the Au NCs is suitable for the detection of HSA. Moreover, molecular platforms capable of performing AND and INHIBIT logic operations are fabricated based on the AEE effect. We expect that this study will provide new insight into protein sensing and offers an experimental basis for the future development of logic devices for molecular information processing and biomedical research.

4. EXPERIMENTAL SECTION

4.1. Materials and Instruments. Hydrogen tetrachloroaurate(III) (≥99.9%), HSA, BSA, ovalbumin, lipase and guanidine hydrochloride were purchased from Sigma. GSH, hemoglobin, horseradish peroxidase, lysozyme, RNase A, carbonic anhydrase, immunoglobulin G, trypsin, and BCA kit were purchased from Sangon Biotech. Phosphate buffers (20 mM) with pH 4.0, 5.0, 6.0, 7.0, 8.0, and 9.0 were prepared by mixing solutions of Na2HPO4 (20 mM) and NaH2PO4 (20 mM). The buffer pH was tuned by HCl/NaOH solution.

Luminescence study was performed on a Shimadzu RF-5301PC fluorometer. Time-resolved luminescence spectra were measured on an Edinburgh FS 920 fluorometer. UV−vis spectra measurement was performed on a Shimadzu UV-1800 spectrophotometer. TEM micrographs were obtained by a FEI Tecnai G2-Twin microscope. XPS were obtained using an ESCALAB-MKII spectrometer. CD spectra were carried out on a Bio-Logic MOS 500 circular dichroism spectrometer. Secondary structural contents were calculated by using the Dichro 2000 program. DLS and ζ-potential measurement were performed on a Brookhaven ZetaPlus apparatus.

4.2. Interactions of GSH−Au NCs with HSA. GSH−Au NCs were prepared following Xie’s method. Typically, 2.0 mL of a HAuCl4 solution (20 mM) was introduced into a solution of GSH (3.3 mM, 18 mL), followed by heating to 70 °C for 24 h. Next, 0.04 mL of the NCs and 100 μL of an aqueous solution of HSA at the required concentration were added to 860 μL of phosphate buffer, followed by incubation for 15 min at ambient temperature prior to any experiment.

4.3. Molecular Logic Operations. 4.3.1. AND Gate. GSH−Au NCs (40 μL) were introduced to 860 μL of phosphate buffer (pH 7.0). The AND gate was implemented upon the introduction of different input combinations into the GSH−Au NC dispersion. (0, 0): H2O; (1, 0): 500 nM HSA; (0, 1): H+, by addition of 0.1 mM HCl to pH 4 and (1, 1): 500 nM HSA and H+ ions.

4.3.2. INHIBIT Gate. GSH−Au NCs (40 μL) were introduced to 860 μL of phosphate buffer (pH 4.0). The INHIBIT gate was implemented upon the addition of different input combinations into the GSH−Au NC dispersion. (0, 0): H2O; (1, 0): 500 nM HSA; (0, 1): OH− ions, by addition of 0.1 mM NaOH to pH 7 and (1, 1): 500 nM HSA and OH− ions.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01875.

UV−vis spectrum, luminescence emission spectrum of GSH−Au NCs (Figure S1); optical images of the Au NC dispersion before and after the addition of HSA (Figure S2); luminescence emission spectra of GSH−Au NCs with and without HSA/GdnHCl (Figure S3); concentration calibration curve in artificial urine (Figure S4); fabrication of INHIBIT logic gate by using ketoprofen (Figure S5); comparison of luminescent methods for serum albumin determination (Table S1); determination of HSA in artificial urine (Table S2) (PDF)

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Notes

The authors declare no competing financial interest.

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