Research Article

Synthesis of Gold Nanoparticles to Capture Lifelike Proteins: Application on the Multichannel Sensor Array Design

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The chemical elements of proteins are similar to that of DNA (e.g., C, H, O, and N), and DNA shows different knotted architectures. So we imagine that proteins may show a wealth of highly complex structures, especially when proteins interact with each other. The imagination was proved by synthesizing gold nanoparticles (GNPs) to capture the lifelike protein structures. The optical responses (i.e., color) of as-prepared GNPs are found to be characteristic to a given protein (or heavy metal ion). Based on the “three colors” principle of Thomas Young, we extracted the red, green, and blue (RGB) alterations of as-synthesized GNPs to fabricate multichannel sensor arrays for proteins (or heavy metal ions) discrimination. The designed multichannel sensor arrays demonstrate possibilities in semiquantitative analysis of multiple analytes (e.g., proteins and heavy metal ions). This work is believed to open new opportunities for GNPs-based label-free sensing.

1. Introduction

Proteins usually fold into one or more specific spatial conformations driven by the noncovalent interactions such as hydrogen bonding, ionic interactions, Van der Waals, and hydrophobic packing [1–3]. Due to the heterogeneous surfaces and numerous spatial configurations, proteins can interact with each other in unpredictable ways [4] and provide opportunities to prepare nanoparticles in the templates of proteins. Interestingly, Taylor comments that protein knots predominantly occur in the catalytic domain or the active site itself [5]. The 2016 Nobel Prize winners, professors Sauvage and Stoddart, predict that advances in the protein area would no doubt continue apace [6]. The chemical elements of proteins are similar to that of DNA (e.g., C, H, O, and N), and DNA shows different knotted architectures [6]. So we imagine that proteins may show a wealth of highly complex structures, especially when proteins interact with each other. To prove such imagination, we designed the experiments to synthesize gold nanoparticles (GNPs) to capture the lifelike protein structures via an in situ reduction method.

Based on this unique optical character, GNPs have been widely employed in colorimetric sensing including metal ions, organic molecules, and proteins [7–10]. The previously reported GNPs-based sensing strategies are mostly designed on the basis of aggregation [11, 12], antiaggregation [13], and etching [14, 15] mechanisms, thus specific designs and complicated surface modification of GNPs being generally inevitable. For example, Naik’s group developed a peptide functionalized GNPs-based colorimetric sensing method for the detection of heavy metal ions [16]. Valentini et al. described a highly sensitive point mutations probe based on DNA-conjugated GNPs [17]. Beyond these, Xia et al. exploited a facile sensing strategy using single-stranded probe DNA, unmodified GNPs, and conjugated polyelectrolyte to detect multiple targets including biomacromolecules (i.e., nucleic acid and protein), small molecules, and metal ions [18]. Nonetheless, the type of analyte is closely dependent upon the kind of selected probe DNA (i.e., aptamer), and thus their strategy could not veritably achieve simultaneous identification and high throughput detection. Consequently,
it is still highly desirable and challenging to develop label-free GNP-based sensing technology and at the same time to attain simultaneous identification of multiple analytes.

Herein, we are interested to develop label-free GNP-based sensing arrays for analytes (e.g., proteins and metal ions) discrimination. We employed the in situ reduction method to synthesize GNP and the “three colors” principle of Thomas Young; that is, “all colors are mixed by red (R), green (G), and blue (B) in different proportions.” We further extracted the RGB data (i.e., fingerprints) from the as-synthesized GNP which is corresponding to a certain analyte and fed them into the Multi-Variate Statistical Package (MVSP) computing software [19]. Then the analytes were quantitatively distinguished by using the statistical methods (e.g., principal component analysis (PCA)) [20]. This current work is believed to open a new strategy for GNP-based sensing without requiring complicated surface functionalization process.

2. Experimental Section

2.1. Materials and Instruments. All chemicals were used as received without further purification. Chloroauric acid tetrahydrate (HAuCl$_4$·4H$_2$O), lysozyme (Lys), trypsin (Try), bovine hemoglobin (BHB), pepsin (Pep), and NaOH were obtained from Sinopharm Chemical Reagent Co., Ltd. (Beijing, China). Collagen (Col), glucoamylase (Glu), and catalase (Cat) were bought from Aladdin Reagent Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA) was purchased from Gibco (Grand Island, USA). 96-well plates (Corning 3632) were obtained from Genetimes Technology. Colorimetric imaging photos were collected by a flatbed scanner (Upson Perfection V300) in 96-well plates. The as-prepared GNP were characterized by transmission electron microscopy (TEM, Tencai F20).

2.2. Preparation of Au(I) Anions. The Col-Au(I) solution was synthesized according to previous reports [21–23]. In brief, HAuCl$_4$ (1 mM) and Col (3 μg/mL) were incubated at room temperature for 10 min and then mixed with NaOH solution (30 mM) under vigorous stirring. Thereafter, the colorless solution of Col-Au(I) was obtained and stored at 4°C for further usage.

2.3. Synthesis of GNP to Capture the Lifelike Protein Structures. In the first experiment, 500 μL of the as-prepared Col-Au(I) solution was mixed with 15 μL proteins (4 mg/mL) and 200 μL H$_2$O$_2$ (5 mM), then rapidly diluted with water to 6 mL and mixed vigorously. In the second experiment, 1 mL of the as-prepared Col-Au(I) solution was mixed with 1 mL Glu (4 mg/mL) and subjected to microwave treatment at 300 W for 90 s. In the third experiment, the NaOH (1 ml, 1M), Glu (50 μL, 2 kU/mL), HAuCl$_4$ (1 mL, 1 mM), and heavy metal ions (20 μL, 80 μM) were mixed vigorously and then subjected to microwave treatment at 300 W for 90 s.

2.4. Multichannel Sensor Array for Protein Discrimination. The colorless Col-Au(I) solution in the absence and presence of proteins and H$_2$O$_2$ were loaded into a 96-well plate, respectively. The flatbed scanner and photoshop software were used to acquire the ARGB alterations of the control (in the absence of proteins and H$_2$O$_2$, denoted as “before”) and the work solutions (in the presence of proteins and H$_2$O$_2$, denoted as “after”). The ARGB data (see Table S1, Electronic Supplementary Material (ESM)) were fed into MVSP (Kovach Computing software) [19]. Then the different proteins were quantitatively distinguished by using the statistical methods (e.g., PCA and and hierarchical clustering analysis (HCA)) [19, 20, 24].

2.5. Multichannel Sensor Array for Heavy Metal Ions Discrimination. The Glu and HAuCl$_4$ mixture in the absence and presence of heavy metal ions were subjected to microwave heating at 300 W for 90 s and then loaded into a 96-well plate, respectively. The flatbed scanner and photoshop software were used to acquire the ARGB alterations of the control (in the absence of heavy metal ions, denoted as “before”) and the work solutions (in the presence of heavy metal ions, denoted as “after”). The ARGB data (see Table S2, ESM) were fed into MVSP and the different heavy metal ions were discriminated by PCA and HCA [19, 20, 24].

3. Results and Discussion

3.1. Synthesis of GNPs to Capture the Lifelike Protein Structures. The chemical elements of proteins are similar to that of DNA (e.g., C, H, O, and N), and DNA shows different knotted architectures [6]. So we imagine that proteins may show a wealth of highly complex structures, especially when proteins interact with each other. To prove such imagination, we designed the experiments to synthesize GNPs in the protein systems via an in situ reduction method. The selected proteins (e.g., Col, Glu, Pep, BHB, Lys, Try, and BSA) are closely related to the life of humans and animals. The multiple interactions among different proteins can induce them showing multiple spatial structures in different environment. It is expected that the multiple spatial structures can be solidified by using strong alkaline or rapid heating. At the same time, the solidified proteins would be covered with GNPs.

The First Experiment. As shown in Figure 1, the GNPs synthesis involves the chemical reduction of Au(III)→Au(I) using Col in the strong alkaline solution and Au(I)→Au(0) in the presence of H$_2$O$_2$ [21, 25, 26], rapidly producing GNPs assembled/overgilt on the protein structures. In detail, Au(III) (i.e., [AuCl$_4$]$^-$, light-yellow) was firstly reduced by Col to obtain Au(I) solution (i.e., [AuCl$_4$]$^-$, colorless) [21–23]. Then seven proteins were selected as testing model to verify the imagination of the lifelike activities of proteins. As shown in Figure S1 (ESM), upon the addition of H$_2$O$_2$ to the mixtures of Col-Au(I) and tested proteins, the colorless solution of Col-Au(I) obviously changed into various colors due to the rapid formation of GNPs on the surfaces of different proteins. It is worth mentioning that the colors of GNPs corresponding to BHB, BSA, and Lys show the different shades of blue (Figure S1, ESM), which means that
Figure 1: Schematic illustration of GNPs synthesis to capture the lifelike protein structures. The multistructures (e.g., irregular porous balls, gears, animal actions, and leaves) may exist in the mysterious protein systems. To verify the imagination, we synthesized GNPs in the proteins solution via the in situ reduction of Au(I) using H$_2$O$_2$. The imaginary structures are formed by simple mixing Pep, Cat, BSA, Glu, and Col, respectively.

The as-synthesized GNPs may be similar to each other or aggregative. To prove the conjectures, we characterized the as-prepared GNPs by TEM. As shown in Figure 2, the GNPs show the different shapes, such as particles dispersed in the irregular porous balls, gears, animal actions, and spheres assembled on leaves. Interestingly, all the shapes of GNPs corresponding to BSA, BHb, and Lys present dog actions, as shown in Figure 2(c) and Figure S2 (ESM). The representative UV-vis absorption spectra were further characterized to reveal the reason for GNPs exhibiting different colors. As shown in Figure S3 (ESM), the UV-vis absorption intensities of as-synthesized GNPs are different and the corresponding peaks appear at ~570 nm (Try), 605 nm (BSA and BHb), and 640 nm (Lys), which means that the produced GNPs are of different concentrations and sizes. The blue coloration of GNPs corresponding to BSA, BHb, and Lys should be more aggregated than the red coloration of GNPs corresponding to Try.

The Second Experiment. By adding H$_2$O$_2$ to the mixtures of Glu and Col-Au(I), we synthesized gold nanospheres on leaves (see Figure 2(d)). In particular, interestingly, the mixture of Glu and Col-Au(I) was then subjected to microwave treatment. The Au(I) ions could be reduced by proteins to be GNPs [21], which might be dispersed on the rolled leaves (as shown in Figure 3(a)). The imagination is proved by the TEM characterization (see Figure 3(b)). The captured structure of Glu-Col (see Figure 3(b)) is similar to the peptide...
configuration reported by professor Feringa (the 2016 Nobel Prize winner) [4].

**The Third Experiment.** Inspired by professor Sauvage’s theory of “In the presence of metal ions, molecules can form the double-stranded double helix” [27], we designed another experiment to capture the life actions in protein systems (see Figure 4). In the strong alkaline solution, [AuCl₄]⁻ ions were mixed with certain heavy metal ions (e.g., Mn²⁺, Co²⁺, Cu²⁺, Cr³⁺, Ni²⁺, Hg²⁺, Zn²⁺, Pb²⁺, and Cr(VI)) at a molar ratio of 625:1 and then mixed with the protein of Glu rapidly. According to our previous reports [21, 23], the metal ions should be reduced to monovalent metal ions (e.g., Au(I), Mn(I), Co(I), and Cu(I)). Livoreil et al. forecasted that the metal-mediated interlocking rings could in principle be extended to polymers [28], and the macromolecular systems incorporating interlocking rings would lead to interesting systems [29]. Here, the monovalent metal ions may mediate the amino acid residues present in Glu to be interlocking rings (i.e., double-stranded double helices) [23, 27]. As shown in Figure S4 (ESM), the strong interactions between monovalent metal ions and the functional groups of Glu (e.g.,
-NH and -CO) help the metal ions in rings to be the cores, which could be deposited by the free metal ions. Through the rapid nucleation method, the metal ions (mainly Au(I) ions) were reduced by the reductive proteins to be GNPs [21]. The animal-hair-shaped GNPs would be synthesized via microwave boiling the helices. The animal actions (see Figure 4(b)) are expected to be captured to synthesize GNPs in the protein systems. The helices containing different distribution and number of various metal ions would result in the synthesized GNPs exhibiting multicolors. The mixtures of metal ions, Au(III) and Glu solution, were subjected to microwave treatment at 300W for 90s, and the GNPs of distinguishable colors were synthesized. By adding Hg$^{2+}$ and Mn$^{2+}$ to the mixture of [AuCl$_4$]$^{-}$ and Glu, we captured the animal actions. As shown in Figure 5(a), a dog is pouncing to the rabbits' hole; the rabbit mother and cub are escaping and leaving their foot prints (see Figures 5(c) and 5(d)). As shown in Figure 5(b), the as-synthesized GNPs are similar to the hairs of animals (e.g., rabbit and dog). The insets of Figure 2, Figure S2 (SI), and Figures 5(b) and 5(d) show that the interplanar distances of GNPs are approximately 0.2 nm, which can be indexed to {200} plane of gold [30]. All the captured protein structures show that the matter, movements, and life actions exist in the biological fields.

3.2. Application on Multichannel Sensor Array Design

**Protein Discrimination.** Encouraged by the distinct colors of GNPs (Figure S1, ESM), we expect that the Col-Au(I) solution might be a promising probe for constructing “single-indicator-based multidimensional sensing” platform that we established recently [31], for the discrimination of proteins. According to the “three colors” principle of Thomas Young, we extracted the RGB alterations of the single-indicator-based sensor images, “before” and “after” exposure to proteins and H$_2$O$_2$ (Figure S1 and Table S1, ESM). The RGB alterations provide diverse fingerprints, as shown in Figure 6(a). The ARGB data are further fed into the MVSP computing software and classified by statistical methods, that is, PCA and HCA [19, 20, 24]. As shown in Figure 6(b), the results of PCA prove that the canonical response patterns of proteins are clearly clustered into different groups, significantly separating from the control. The results of HCA show that all of the 40 cases (8 samples × 5 replicates) are correctly assigned to their respective groups (Figure S5, ESM). The constructed platforms provide a new way to detect the proteins contained in the complex matrix (e.g., milk), showing great promise for milk safety in the future.

**Heavy Metal Ions Discrimination.** As shown in Figure 7, from the color images of the [AuCl$_4$]$^{-}$ and Glu mixture (as indicator) in the absence (named “before”) and presence (named “after”) of heavy metal ions after microwave reaction, the obvious color differences are observed to the target heavy metal ions. The visual sensor array seems to be successful at differentiating the heavy metal ions (0.77 $\mu$M) as shown in the difference maps, which are obtained through taking the RGB variations from the “before” and “after” images. The unique pattern of difference maps is corresponding to a certain heavy metal ion (see Figure 7). The observations demonstrate that the single-indicator-based sensor can potentially determine the target heavy metal ions (0.77 $\mu$M) via constructing the multichannel visual sensing array. The RGB alterations (Table S2, ESM) provide diverse fingerprints (see Figure 8(a)), further fed into MVSP computing software and classified by PCA [19, 20]. The canonical colorimetric response patterns (3 channels × 10 samples × 4 replicates) are clustered into several groups (see Figure 8(b)). The resulting 2D canonical score plot (Figure 8(b)) shows clear clustering of the data using the first two principal components (representing 97% of the total variance), with excellent discriminatory capacity. The PCA plots for various
Figure 5: (a, b) The captured GNPs’ TEM images of animal actions and (c, d) foot prints at different magnification. Insets: the interplanar distances of GNPs determined by using ImageJ software.

Figure 6: (a) ΔRGB alterations of the Col-Au(I) solution in the absence and presence of seven proteins (10 μg/mL) and H₂O₂. Each value is an average of five parallel measurements, and the error bars are shown. (b) The seven proteins were quantitatively distinguished by the PCA method.
heavy metal ions are not random but rather follow certain patterns. As shown in Figure 8(b), all the target heavy metal ions (0.77 μM) are separated from each other, demonstrating that they are effectively discriminated by PCA based on both the ΔRGB values (Table S2, ESM). The single indicator in the presence of the nine heavy metal ions at 0.77 μM are also analysed using HCA [19, 24]. All of the 40 cases (10 samples × 4 replicates) are correctly assigned to their respective groups (Figure S6, ESM). The constructed platform provides a new way to detect the heavy metal ions contained in the environmental water or air, showing great promise for environmental safety in the future.

### 4. Conclusion

In summary, the chemical elements of proteins are similar to that of DNA (e.g., C, H, O, and N), and DNA shows different knotted architectures. So we imagined that proteins might show a wealth of highly complex structures, especially when proteins interact with each other. The imaginations have been proved by the experiments of synthesizing GNPs to capture the lifelike protein structures.

Moreover, a novel and facile sensing strategy based on in situ reduction has been developed. The main advantage of this strategy is label-free, which avoided frequently used surface modification in most GNPs-based sensors. The as-proposed sensing method is able to identify and detect multiple analytes (e.g., proteins and heavy metal ions). This work is believed to open new opportunities for fabricating GNPs-based label-free, simultaneous, and high throughput identification of multiple targets in samples (e.g., milk and environmental water).

### Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.
Authors’ Contributions

Yumin Leng conceived the idea, performed the experiments, analysed the data, and wrote the manuscript. Wentai Zhang drew Figures 1, 3(a), and 4. Kecheng Liu characterized GPNPs. Chunhua Ma and Zhiwen Lu analysed the data and discussed the paper. All authors have read and approved the manuscript.

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Supplementary Materials

Figure S1: Color images of the Col-Au(I) solution obtained before and after exposure to proteins and H2O2. The randomly selected proteins (10 μg/mL) based on the modified Au(I) to GPNPs, which are overgilt on the protein structures (e.g., BSA, lysozyme (Lys), glucoamylase (Glu), and trypsin (Try)). Figure S2: TEM and high resolution TEM (HRTEM) images of GPNPs obtained by the rapid reduction of Au(I) to GPNPs, which are overlain on the protein structures (e.g., BHB and Lys interacted with Col). Figure S3: The UV-vis absorption spectra of GPNPs obtained by the in situ reduction of Au(I) to GPNPs in the protein systems (the signed proteins interacted with Col). Figure S4: Schematic illustration of the interlocking rings directed by monovalent metal ions (M(I)) in Glu. Figure S5: HCA analysis for the discrimination of proteins (10 μg/ml) based on the ΔRGB variations. Figure S6: HCA analysis for the discrimination of heavy metal ions (0.77 μM) based on the ΔRGB variations. Table S1: Database of the ΔRGB alterations of the Col-Au(I) solution in the absence and presence of seven proteins (10 μg/ml) and H2O2. Table S2: Database of the ΔRGB alterations of the [AuCl4]− and Glu mixture in the absence and presence of heavy metal ions (0.77 μM) after microwave reaction. (Supplementary Materials)

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