Fluorosurfactant-capped gold nanoparticles-based label-free colorimetric assay for Au$^{3+}$ with tunable dynamic range via a redox strategy

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**A B S T R A C T**

Gold nanoparticles-based colorimetric assay possesses several unique advantages, and has been applied for a wide range of targets, varying from nucleic acids to different metal ions. However, due to the lack of proper coordinating ligand, gold nanoparticles-based colorimetric sensing system for Au$^{3+}$ has not been developed so far. It is well-known that Au$^{3+}$ could induce the oxidation transition of thiol compounds to disulﬁde compounds. In this article, for the first time we converted such thiol masking reaction into colorimetric sensing system for label-free detection of Au$^{3+}$ via a target-controlled aggregation of nanoparticles strategy. In the new proposed sensing system, fluorosurfactant-capped gold nanoparticles were chosen as signal reporter units, while an Au$^{3+}$-triggered oxidation of cysteine (Cys), which inhibited the aggregation of gold nanoparticles, acted as the recognition unit. By varying the amount of Cys, a tunable response range accompanied with different windows of color change could be obtained for Au$^{3+}$, illustrating the universality of the sensing system for Au$^{3+}$ samples with different sensitivity requirements. Under optimized condition, the proposed sensing system exhibits a high sensitivity towards Au$^{3+}$ with a detection limit of 50 nM, which is lower than previously reported spectroscopic methods. It has also been applied for detection of Au$^{3+}$ in practical water samples with satisfactory result.

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1. Introduction

Besides the widespread monetary and symbolic functions, gold also has many practical uses due to its unique physical and chemical properties, and is very useful in pollution control, mobile phones, laptops, space travel, dentistry, and many others (Ali and Christie, 1984; Pattou et al., 1996; Apilux et al., 2010). Moreover, some gold complexes have been utilized as effective drugs in treating rheumatoid, psoriatic arthritis and bronchial asthma for 80 years (Frank Shaw, 1999; Sun and Che, 2009; Casini et al., 2006). However, gold ions are also known to be highly toxic in the biological systems because they can strongly bind to DNA to cause its cleavage (Habib and Tabata, 2004; Nyarko et al., 2004). Therefore, the development of methods for efficient detection of Au$^{3+}$ in various samples is of considerable significance, and has become an attractive subject of modern analytical chemistry. As a consequence, several methods for the detection of gold ions at trace quantity level in various samples have been proposed. They include atomic absorption spectroscopy (AAS) (Gabrel and Law, 1983), atomic emission spectroscopy (Falkner and Edmond, 1990), inductively coupled plasma quadrupole mass spectrometry (ICP-MS) (Pitcairn et al., 2006), and so on. In addition to these sophisticated apparatus-dependent techniques, probing technology, which is much simpler in instrumental implementation and sample pretreatment, was also developed for Au$^{3+}$ determination. In the past decades, considerable attention has been focused on the design of organic small molecule-based fluorescent probes (Jou et al., 2009; Yang et al., 2009; Park et al., 2012; Cao et al., 2011) or colorimetric probes (Clem and Huffman, 1965; Lichtenstein, 1975; Kamble et al., 2010; Jang and Roper, 2011) for Au$^{3+}$. However, most of these organic small molecules are not commercially available and generally need tedious and time-consuming synthesis, moreover some of them are water-insoluble and require the addition of organic co-solvents which might limit their practical applications.

Gold nanoparticles (AuNPs) possess unique size/distance-dependent optical properties, very high extinction coefficients and photostability, and are ease of functionalization with sensing moiety, and therefore, are ideal candidates for developing colorimetric probes (Jain et al., 2006; Boisselier and Astruc, 2009; Saha et al., 2012; Zayats et al., 2005). AuNPs-based colorimetric probes...
have received considerable attention in chemical and biological analysis because of their simplicity, high sensitivity, and low cost. Moreover, such probes may minimize or even eliminate the use of analytical instruments, and can easily realize on-site detection. In the past two decades, quite a few AuNPs-based colorimetric probes have been developed for a wide range of targets, including nucleic acids (Elghanian et al., 1997; Li and Rothberg, 2004), small molecules (Zhao et al., 2008; Jung et al., 2011; Jiang et al., 2010), proteins (Guarise et al., 2006), cells (Medley et al., 2008) and so forth. Such probes for metal ions such as Hg2+ (Xue et al., 2008; Darbha et al., 2008; C.Y. Lin et al., 2010), Pb2+ (Liu and Lu, 2003), Cu2+ (Qu et al., 2011), Ag+ (Hung et al., 2010; C.Y. Lin et al., 2010), Ca2+ (Kim et al., 2009), Ln3+ (Lisowski and Hutchison, 2009), have also been reported via a specific ligand–metal ion complexation strategy or a metal deposition mechanism. Despite considerable recent interest and advances in gold ions detection research, there have been no reports on AuNPs-based colorimetric probes for Au3+ due to the lack of proper coordinating ligand.

Among abovementioned colorimetric probes, label-free type probes have attracted particular attention since no special chemical modification is necessary for AuNPs (Li and Rothberg, 2004; Jung et al., 2011; Jiang et al., 2010; C.Y. Lin et al., 2010; Hung et al., 2010). However, these probes are usually based on AuNPs coated with an ionic compound such as citrate to stabilize their dispersion in buffered solution, which might be affected by high ionic strength or changed pH values, and strictly limit their application environments. More recently, a kind of AuNPs capped with nonionic fluorosurfactant molecules (FSN-AuNPs) has received increasing attention as the FSN moiety can stabilize AuNPs against conditions of high ionic strength and a wide pH range, while free thiol groups can replace FSN molecules from the Au surface and increasing attention as the FSN moiety can stabilize AuNPs against conditions of high ionic strength and a wide pH range, while free thiol groups can replace FSN molecules from the Au surface and trigger the AuNPs rapid aggregation through the London-van der Waals attraction force. Such a unique feature was then adopted to construct colorimetric probe for Cys (Lu et al., 2007; Lu and Zu, 2007; Xiao et al., 2011), homocysteine (Huang and Tseng, 2008; J.-H. Lin et al., 2010) and its relative enzyme activity (J.H. Lin et al., 2010). It is well-known that Au3+ could induce the oxidation transition of thiol compounds to disulfide compounds to mask the free thiols (Frank Shaw et al., 1979; Moustathis and Garnier-Suillerot, 1989; Brinas et al., 2008). Herein, by combining such Au3+-triggered thiol masking reaction with the unique features of FSN-AuNPs, for the first time we reported a colorimetric sensing system for rapid and label-free detection of Au3+ (Fig. 1).

2. Materials and methods

2.1. Materials and instruments

TPEN (N, N, N, N-tetraakis-(2-pyridylmethyl)-ethylenediamine) and FSN were obtained from Sigma-Aldrich (China). The molecular formula of FSN is \( F(CF_2CF_2)_3CH_2CH_2O(CH_2CH_2O)_xH \). Hydrogen tetrachloroaurate(III) tetrahydrate, l-cysteine (Cys) and other chemicals were purchased from Shanghai Chemical Reagent Corporation (Shanghai, China). Water used in all experiments was doubly distilled and purified by a Milli-Q system (Millipore, USA).

The absorption spectra of the FSN-AuNPs were measured using a UV-2450 spectrophotometer (Shimadzu). Dynamic light scattering (DLS) was performed on a Zeta Sizer Nano ZS. Transmission electron microscopy (TEM) images were obtained by using a JEOL1400 model at an accelerating voltage of 100 kV.

2.2. Preparation of FSN-AuNPs

AuNPs with average diameters of 13 nm were prepared following the literature procedure (Li et al., 2010). Briefly, 0.75 mL of 1% HAuCl4 in 99 mL of water was heated to reflux, and then a 6 mL solution of 1% sodium citrate was rapidly added. The solution was heated under reflux with vigorous stirring for about 15 min until its color changed from pale yellow to deep red. After heated with vigorous stirring for another 30 min, the solution was cooled to room temperature with a continuous stir for 2 h. The concentration of the AuNPs was calculated to be about 3.0 nM by assuming spherical AuNPs was concentrated by a factor of 8 and then FSN (10%) was added to get a final concentration of 0.4%. The resulting mixture was stored at 4 °C until further use.

2.3. Colorimetric assay of Au3+

Twenty microliter of various amounts of Au3+ was first added into 60 µL of buffer (200 mM PBS, 0.1 M NaBr, 0.2 mM TPEN), 20 µL...
of 12, 24, 36, or 48 μM Cys was then added to this solution. 1 min later, the resulting solutions were mixed with 20 μL 24 nM FSN-AuNPs. The final solutions were equilibrated at room temperature for 2 min, and then the UV–vis absorption spectra of the solution were recorded immediately. The selectivity of our colorimetric assay was tested under identical conditions in the presence of other transition metal ions, including Cd²⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Zn²⁺, Cr³⁺, Pb²⁺, Cu²⁺, Hg²⁺, while TPEN in the buffer was removed to examine the response in the presence of Ag⁺.

2.4. Determination of Au³⁺ in diver and spring water

Samples of river water and spring water were collected from Xiang River and Yuelu Mountain (Changsha, China), respectively, and used after simple filtration. Aliquots of the river water or the spring water (1 mL) were spiked with standard Au³⁺ solutions and stored at room temperature for 1 h. Finally, the samples were analyzed according to the general procedure for Au³⁺ determination described above. For Au³⁺ with final concentration of 0.5 and 2.0 μM, 20 μL of 24 μM and 48 μM Cys was used as probe.

3. Results and discussion

3.1. Design and working mechanism

In our new designed sensing system (Fig. 1), FSN-AuNPs act as signal reporter units, while Cys serves as a molecular recognition module for Au³⁺. In the absence of Au³⁺, the free thiol of Cys can replace FSN molecules from the Au surface due to the strong Au-thiol affinity, and trigger the AuNPs rapid aggregation through the London-van der Waals attraction force (Lu et al., 2007). As shown in Fig. 2, addition of 8 μM Cys induced the red-shift change of FSN-AuNPs’ surface plasmon resonance (SPR) band, accompanied by a characteristic red-to-blue color change (inset in Fig. 2A), indicating the occurrence of AuNPs aggregation. The introduction of Au³⁺, however, will trigger the oxidation of Cys to cystine which is difficult to bind on the surface of FSN-AuNPs due to lack of free thiol groups (Lu et al., 2007), thereby inhibiting the aggregation of AuNPs even in a high ion-strength solution. The incubation of 3 μM Au³⁺ with 8 μM Cys for 1 min could inhibit the red-shift change of FSN-AuNPs’ SPR band. At the same time, the color of the solution remained pink red (inset in Fig. 2A).

Both TEM images (Fig. 2B) and dynamic light scattering spectra (see Fig. S1 in the ESI) confirmed that in the presence of Au³⁺ the aggregation of FSN-AuNPs driven by Cys was completely suppressed, which was consistent with the abovementioned UV–vis absorption spectra results. A UV–vis spectroscopic investigation of the mixture of Au³⁺ and Cys in a 1:5 M ratio showed a new absorption at 320 nm (see Fig. S2 in the ESI). This band could be assigned to dₓ−pₓ transition of Au(I) complexes, indicating the formation of Au(I)-Cys (Brinas et al., 2008).

3.2. Selectivity of the sensing system

Selectivity is a very important parameter to evaluate the performance of a new sensing system with potential application in practical samples. However, due to the high affinity of thiol group towards several transition metal ions, it is a challenge for our colorimetric sensing system to selectively detect Au³⁺ over other metal ions (Bjerrum et al., 1957; Liu et al., 2010). Therefore, the selectivity experiments for the sensing system were extended to various metal ions, including Cd²⁺, Mn²⁺, Fe³⁺, Co²⁺, Ni²⁺, Zn²⁺, Cr³⁺, Pb²⁺, Cu²⁺, Hg²⁺, Ag⁺. These metal ions were first mixed with Cys separately, and the absorption intensity ratios at 650 nm and 520 nm of the mixture (A₆₅₀ nm/A₅₂₀ nm) were then recorded after the addition of FSN-AuNPs (Fig. 3). In the presence of classical masking agents such as TPEN and NaBr, this sensing system showed good selectivity to Au³⁺ over other metal ions, which is due to the reason that the stability constant of Cys toward these metal ions (log Kₛ < 20) is lower than that toward AuNPs (log Kₛ = 30). In such a way, the AuNPs here not only severed as a signal reporter moiety for colorimetric detection, but also could act as a “nano-competing agent” to improve the selectivity of the sensing system.

3.3. Sensitivity and dynamic range

To evaluate the sensitivity of the sensing system, the aggregation performance of the FSN-AuNPs induced by Cys was first investigated. Owing to the replacement of FSN from the surface of AuNPs, Cys can induce the aggregation process of FSN-AuNPs within 2 min, with a moderate response range of 1–6 μM (see Fig. S3 in the ESI). A post-mixing approach was then applied to detect Au³⁺, i.e., 4 μM of Cys was incubated with various amounts of Au³⁺ for 1 min before the addition of FSN-AuNPs, which inhibited the aggregation of FSN-AuNPs due to the decreased concentration of free Cys. With the concentrations of Au³⁺ increasing,
the color of the solutions changed gradually from purple to red, and the absorption intensity at 520 nm gradually increased along with decreasing absorption intensity at 650 nm (Fig. 4A). The value of \( A_{520 \text{ nm}}/A_{650 \text{ nm}} \) is linear with the \( \text{Au}^{3+} \) concentrations within a range from \( 1.0 \times 10^{-7} \) to \( 1.2 \times 10^{-6} \) M (Fig. 4B), with a detection limit of \( 5.0 \times 10^{-8} \) M (3σ/slope) which is lower than the previously reported spectroscopic methods (Jou et al., 2009; Yang et al., 2009; Park et al., 2012; Lichtenstein, 1975; Kamble et al., 2010; Jang and Roper, 2011). The ratio of the \( \text{Au}^{3+} \) concentration at the complete recovery of the absorption of FSN-AuNPs to the initial concentration of Cys was about 1:3, which was also consistent with the mechanism above that \( \text{Au}^{3+} \) mainly reacted with Cys in a stoichiometric way in a 1:3 M ratio (Fig. 1).

A tunable dynamic range is important for a sensing system to be applied in practical samples as the desirable concentrations for the target analyte widely depends on the environments it existing in (Pu et al., 2010; Kim et al., 2011; Zhou et al., 2011; Luo et al., 2011). We try to adjust the concentration of Cys to tune the response range for the sensing system. With the concentration of Cys fixed at 2 \( \mu \)M, a dynamic response concentration range from \( 1.0 \times 10^{-7} \) to \( 5 \times 10^{-7} \) M was observed for \( \text{Au}^{3+} \) (Fig. 5A). In the presence of 4 \( \mu \)M Cys, the dynamic range was shifted to \( 1.0 \times 10^{-7} \)– \( 1.2 \times 10^{-6} \) M (Fig. 5B). When the concentration of Cys increased to 6 \( \mu \)M and 8 \( \mu \)M, the dynamic range was \( 0.8 \times 10^{-6} \)– \( 1.6 \times 10^{-6} \) M (Fig. 5C) and \( 1.5 \times 10^{-6} \)– \( 2.5 \times 10^{-6} \) M (Fig. 5D) respectively. In this way, the dynamic range of this colorimetric probe can be tuned easily by varying the concentrations of Cys while other detection procedures are similar.

Table 1

<table>
<thead>
<tr>
<th>Sample</th>
<th>( \text{Au}^{3+} ) spiked (( \mu )M)</th>
<th>( \text{Au}^{3+} ) recovered mean ( \pm ) SD</th>
<th>Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>River water 1</td>
<td>0.5</td>
<td>0.48 ( \pm ) 0.01</td>
<td>96.0</td>
</tr>
<tr>
<td>River water 2</td>
<td>2.0</td>
<td>1.93 ( \pm ) 0.09</td>
<td>96.5</td>
</tr>
<tr>
<td>Spring water 1</td>
<td>0.5</td>
<td>0.52 ( \pm ) 0.01</td>
<td>104.0</td>
</tr>
<tr>
<td>Spring water 2</td>
<td>2.0</td>
<td>1.95 ( \pm ) 0.11</td>
<td>97.5</td>
</tr>
</tbody>
</table>

* Mean of three determinations.  
 SD: standard deviation.

3.4. Detection of \( \text{Au}^{3+} \) in real samples

The practical application of the sensing system was then evaluated by determination of recovery of spiked \( \text{Au}^{3+} \) in river and spring water samples. The river water samples were obtained from Xiang river, and spring water samples were obtained from Yuelu mountain (Changsha, China). All the samples collected were simply filtered and showed that no \( \text{Au}^{3+} \) was present. \( \text{Au}^{3+} \) stock solution at different concentrations was spiked in these samples, and the abovementioned post-mixing approach was then employed to detect its concentration, with analytical results shown in Table 1. It was observed that the results obtained in real water samples show good recovery values, which confirmed that the proposed sensing system was applicable for practical \( \text{Au}^{3+} \) detection in real samples with other potentially competing species co-existing.

4. Conclusions

In summary, we have developed a sensitive and colorimetric sensing system for \( \text{Au}^{3+} \) based on the fact that Cys can trigger the rapid aggregation of FSN-AuNPs in the presence of a high concentration of salt, while its oxidized product-cystine induced by \( \text{Au}^{3+} \) will inhibit the aggregation of FSN-AuNPs due to the lack of a free thiol group. More attractively, by simply varying the amount of Cys, the response range and the color change windows of AuNPs towards \( \text{Au}^{3+} \) can be tuned in a range from 100 nM to 2.5 \( \mu \)M, with a detection limit of 50 nM. Because all the reagents such as Cys, FSN and AuNPs are commercially available, and either colorimetric assay or UV–vis absorption determination obviates the need of expensive equipments, this method is low-cost, simple in design.
and preparation, and can be easily carried out by simple mixing and incubation. It has also been successfully applied for practical Au\textsuperscript{+} detection in environmental water samples, further demonstrating its value in the practical applications.

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**Appendix A. Supporting information**

Supplementary information associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.bios.2013.03.044.

**References**