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Surface plasmon resonance additivity of gold nanoparticles for colorimetric identification of cysteine and homocysteine in biological fluids

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ABSTRACT

In this work, with the assistance of the additivity of surface plasmon resonance band of nonionic fluorosurfactant-stabilized gold nanoparticles, we developed a new colorimetric assay approach of cysteine (Cys) and homocysteine (Hcy) in biological fluids, requiring no use of separation techniques. The detection limits of Cys and Hcy can be as low as 0.4 μM. The applicability of the method was validated by spiking known amount of Cys and Hcy in human urine and plasma samples. Recoveries were in the range of 97.2–106.7%. The present approach is simple, high selective and reproducible. In addition, the universality of the additivity of surface plasmon resonance band was demonstrated by virtue of silver nanoparticles. Therefore, the proposed method has a great potentiality in disease diagnosis associated with Cys and Hcy.

1. Introduction

Gold nanoparticles exhibit colloidal surface plasmon resonance (SPR), a phenomenon caused by the collective oscillations of surface electrons induced by visible light. The plasmon resonance band of gold nanoparticles can be modulated by the size, shape and composition of the nanoparticles, the distance between nanoparticles, and the refractive index of the surrounding medium. The aggregation of gold nanoparticles induces interparticle coupling of plasmons, accompanying red-to-blue color change [1–3]. In 1997, Mirkin's group pioneered in the use of the distance-dependent optical properties of gold nanoparticles for selective colorimetric detection of DNA [4]. Hereafter, optical measurements based upon SPR have become increasingly popular and widely applied for absorption-based colorimetric sensing of any target that directly or indirectly causes the gold nanoparticle aggregation [5–7]. There exists two strategies to achieve gold nanoparticle-based selective colorimetric sensing of any target: surface functionalization of gold nanoparticles and separation techniques [8,9].

For a solution containing two or more colored components, which do not interact with each other, their absorbances are additive. The principle is well known as the additivity of individual absorbance, meaning that the absorbance of the reaction mixture at a specific wavelength is the sum of the absorbance of the individual components in the reaction mixture at that wavelength [10,11]. According to Beer's Law, which states that $A=\varepsilon_\lambda bc$, where A is the absorbance of the individual component at the wavelength λ, b is the optical path length, c is the concentration of the individual component, and ε_λ is the molar extinction coefficient of individual component at the wavelength λ, whose value is obtained from absorbance measurement of pure solution of the individual component at the designated wavelength. The absorbance additivity is given by $A=\varepsilon_{\lambda 1}bc_1+\varepsilon_{\lambda 2}bc_2+\cdots+\varepsilon_{\lambda n}bc_n$. The additivity of individual absorbances has been applied to the quantitative analysis of multicomponent mixtures [12,13].

Cysteine (Cys) and homocysteine (Hcy) are widely occurring aminothiols with similar structure and properties. There is a metabolic relation between Cys and Hcy in biological fluids, and Hcy can be catabolized to Cys through the transsulfuration pathway [14]. Levels of Cys and Hcy in biological fluids are related to several human diseases. Therefore, accurate identification of Cys and Hcy in biological fluids is vital in disease diagnosis and in therapeutic drug monitoring. However, owing to only one methylene difference in structure of Cys and Hcy, it is very hard to distinguish from each other without chromatographic separation [15–17].

In 2007, Zu's group found that Cys and Hcy can induce a redshift in SPR band of nonionic fluorosurfactant-functionalized gold nanoparticles; however, little spectral change occurred in the presence of other standard amino acids, cystine, homocystine, glutathione and cysteinylglycine (Figs. S1–S4) [18]. Subsequently,

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the colorimetric detection of Cys and Hcy in biological fluids has been successfully developed by tuning the gold nanoparticle size and the pH values of the gold colloidal solution [19,20]. However, the previous papers [18–20] have several limitations such as the following ones: (1) sophisticated separation equipment [18]; (2) AuNPs of two sizes (12 and 40 nm) need to be prepared, and longer aggregation time for the 40 nm AuNPs (20 min) was required [19]; and (3) the pH values of gold colloidal solution require tuning [20]. In this work, these obstacles are overcome by utilizing the additivity of individual SPR band. Interestingly, it is found that Cys and Hcy can be well identified when we choose the two suitable wavelengths from the SPR band of nonionic fluorosurfactant-functionalized gold nanoparticle aggregation induced by addition of a mixture of Cys and Hcy. The proposed approach has been successfully applied to determine Cys and Hcy in human urine and plasma samples with simple procedure and shorter response time. To the best of our knowledge, this is the first example to combine SPR band of gold nanoparticles with the absorbance additivity.

2. Experimental

2.1. Reagents

Zonyl FSN-100 ($F(CF_2CF_2)_{1-7}CH_2CH_2O(CH_2CH_2O)_{0-15}H$), tris(2-carboxyethyl) phosphine (TCEP, ≥98.0%), ethylenediaminetetraacetic acid (EDTA, > 99%), 18 standard amino acids (≥99.0%), homocysteine (> 90%) and glutathione (> 98%) were purchased from Sigma-Aldrich. Hydrogen tetrachloroaurate(III) trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.99%), cysteine (> 99%) and trisodium citrate (99%) were purchased from Acros (Geel, Belgium). Silver nitrate (99%) was purchased from J&K Scientific Ltd. Sodium phosphate dibasic anhydrous (99.0%) and sodium phosphate monobasic anhydrous (≥99.0%) were purchased from Tianjin Fucheng Chemical Reagent Company, China. Perchloric acid (70–72%), sodium chloride (≥99.5%) and nitric acid (63–68%) were purchased from Beijing Chemical Reagent Company, China. The pH of the phosphate buffer solution (PBS) was adjusted with NaOH or HCl. 10 mM stock solutions of Cys and Hcy were freshly prepared by dissolving their commercial crystal in deionized water and stocked at 4 °C, and their working solutions were freshly diluted with deionized water. All solutions were prepared with deionized water (Milli Q, Millipore). All reagents were of analytical grade and used without further purification.

2.2. Apparatus

The UV–visible (UV–vis) absorption spectra were measured at room temperature on a Shimadzu UV-2401 PC spectrophotometer (Tokyo, Japan) with a variable wavelength between 400 and 800 nm using a glass cuvette with 1.0 cm optical path. The size, shape and their distribution of gold nanoparticles were confirmed through transmission electron microscope (TEM) measurements using a Tecnai G220 TEM (FEI Co., Netherlands) at an accelerating voltage of 200 kV. The TEM specimens were prepared by depositing an appropriate amount of gold nanoparticles onto the carbon-coated copper grids and excess solution was wicked away by a filter paper. The grid was subsequently dried in air before measurement.

2.3. Preparation of gold nanoparticles

All glassware for preparation of gold nanoparticles were thoroughly cleaned with freshly prepared aqua regia (HNO_3:HCl = 1:3), rinsed extensively with deionized water, and then dried in an oven at 100 °C for 2–3 h. The gold nanoparticles were prepared according to the literature [18]. In brief, a 50 mL solution of 0.04% sodium citrate was brought to a vigorous boil with stirring in a round-bottom flask with a reflux condenser for 2 min, and then 85 μL of 5% $HAuCl_4$ was added to the stirring and refluxing sodium citrate solution. The solution was maintained at the boiling point with continuous stirring for another 15 min. The color of the solution turned to wine-red. After the solution was cooled to room temperature under continuous stirring, 400 μL of 5.0% nonionic fluorosurfactant was added under stirring overnight. The gold colloidal solution was stored at 4 °C before use.

2.4. Preparation of silver nanoparticles

The silver colloidal solution was prepared according to the reported method with minor modification [21]. Briefly, a 50 μL solution of 0.1 M $AgNO_3$ and a 50 μL solution of 0.1 M trisodium citrate were added into 20 mL deionized water with stirring in a round-bottom flask. Then, 1.2 mL of freshly prepared 25 mM sodium borohydride ice-chilled solution was added into the above aqueous solution drop by drop with vigorous stirring at room temperature. Stirring was stopped after 30 min, and pale yellow color marked the end of the reaction. Then, 172 μL of 5.0% nonionic fluorosurfactant was added under stirring overnight. The silver colloidal solution was stored at 4 °C before use.

2.5. Procedures for detection of cysteine and homocysteine

For detection of Cys and Hcy at the same time, the pH value of nonionic fluorosurfactant-capped gold colloidal solution was 6.5. 0.95 mL nonionic fluorosurfactant-capped gold colloidal solution (pH 6.5) in 100 mM NaCl was incubated in a thermostat water bath at 50 °C for 30 s in a quartz cuvette, followed by the addition of different concentrations of Cys/Hcy working or sample solution. The resulting solution was monitored by the UV–vis spectrophotometer. The UV–vis absorption spectra were recorded after 30 s incubation at 50 °C by scanning the UV–vis spectrophotometer from 400.0 nm to 800.0 nm.

The pH of nonionic fluorosurfactant-capped silver colloidal solution was adjusted with HCl to 4.7. 10 mM stock solution of Cys/Hcy was diluted into 100 μM, and 0.95 mL nonionic fluorosurfactant-capped silver colloidal solution (pH 4.7) in 70 mM NaCl was thoroughly mixed with different concentrations of Cys/Hcy in a quartz cuvette. The UV–vis absorption spectra were recorded after 1.0 min incubation time by scanning the UV–vis spectrophotometer from 300.0 nm to 700.0 nm at room temperature.

2.6. Pretreatment of human urine and plasma samples

Human urine samples were collected from healthy humans, and the analysis was conducted immediately after the sample collection. Human plasma samples were obtained from China–Japan Friendship Hospital (Beijing, China). The standard addition was carried out by spiking a certain amount of Cys/Hcy standard solution to urine or plasma samples. To 100 μL of urine or plasma sample in a centrifuge tube, 50 μL of 0.1 M EDTA and 5.0 μL of 0.2 M TCEP in pH 6.0 PBS were added. The mixture was kept at 60 °C for 30 min. After cooling to room temperature, the sample was gently vortex-mixed with 20 μL of 3.0 M perchloric acid solution, put aside at room temperature for 10 min, and then centrifuged at 13,000 rpm for 10 min. The clear supernate was filtered through a 0.22-μm filter and diluted when required before analysis.

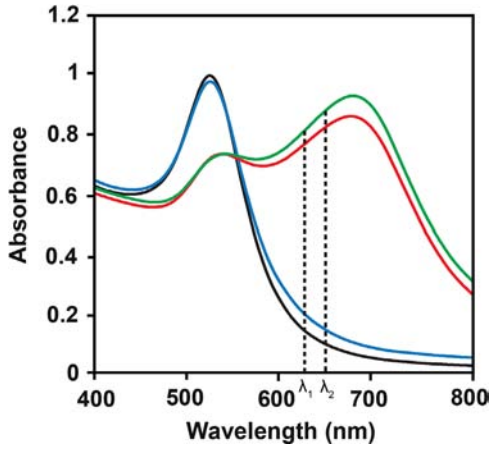

Fig. 1. UV–vis absorption spectra of nonionic fluorosurfactant-capped gold nanoparticles in the presence of no analyte (black), 4.0 μM Cys (red), 0.5 μM Hcy (blue), or the mixture of 4.0 μM Cys and 0.5 μM Hcy (green). λ_1 and λ_2 represent 630 nm and 650 nm, respectively. The nonionic fluorosurfactant-capped gold colloidal solution at pH 6.5 contained 100 mM NaCl with 30 s incubation period at 50 °C, and the absorption values were measured at 30 s after addition of 4.0 μM Cys, 0.5 μM Hcy, and the mixture of 4.0 μM Cys and 0.5 μM Hcy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3. Results and discussion

3.1. Additivity of individual surface plasmon resonance

Nonionic fluorosurfactant-capped gold nanoparticles possess high extinction coefficient, and thus it is possible to develop the additivity of individual surface plasmon resonance of gold nanoparticles. In the current approach, under the optimal conditions (i.e., 2.9 nM nonionic fluorosurfactant-capped gold colloidal solution in 100 mM NaCl with 30 s incubation at 50 °C), we can write the following expression assuming a unit path length (1.0 cm):

$$A_{\lambda 1} = \varepsilon_{\lambda 1/\mathrm{Cys}} c_{\mathrm{Cys}} + \varepsilon_{\lambda 1/\mathrm{Hcy}} c_{\mathrm{Hcy}}$$

$$A_{\lambda 2} = \varepsilon_{\lambda 2/\mathrm{Cys}} c_{\mathrm{Cys}} + \varepsilon_{\lambda 2/\mathrm{Hcy}} c_{\mathrm{Hcy}}$$

As shown in Fig. 1, analyte-induced absorbance change at 630 nm is 0.052 for 0.5 μM Hcy, 0.666 for 4.0 μM Cys, and 0.714 for the mixture of 0.5 μM Hcy and 4.0 μM Cys. After the addition of the absorbance values of 0.5 μM Hcy and 4.0 μM Cys, the absorbance value obtained can be 0.718. As a result, the relative error is 0.6% at 630 nm. Similarly, the changes in the absorption spectra at 650 nm are 0.046 for 0.5 μM Hcy, 0.749 for 4.0 μM Cys, and 0.807 for the mixture of 0.5 μM Hcy and 4.0 μM Cys. The relative error is 1.5% at 650 nm. It can be concluded that the absorbance changes of the gold nanoparticles aggregation induced by Cys and Hcy obey the additivity of individual absorbances. Moreover, the absorbance changes of nonionic fluorosurfactant-capped gold nanoparticles at all wavelengths from 610 to 690 nm were investigated in the presence of 4.0 μM Cys, 0.5 μM Hcy or a mixture of 4.0 μM Cys and 0.5 μM Hcy (Fig. 2). The relative errors can range from 0.4% to 4.3%, indicating good accuracy. In conclusion, the additivity of individual absorbance of nonionic fluorosurfactant-capped gold nanoparticles can be used for sensing Cys and Hcy.

3.2. Molar extinction coefficients

The values of the molar extinction coefficients are very important for this experiment. In this work, the values of the molar extinction coefficients for Cys and Hcy at 630 and 650 nm can be obtained from absorbance measurements of the standard solutions of Cys and Hcy at the designated wavelengths (630 and 650 nm). Within the linear ranges of Cys and Hcy from 0.5 μM to 4.5 μM, we calculate the molar extinction coefficients for Cys and Hcy at 630 and 650 nm according to the Beer–Lambert law. Table 1 indicates that the values of the molar extinction coefficients are large enough for sensitive detection of Cys and Hcy, and the standard deviation (SD) for all the molar extinction coefficients is below 0.003.

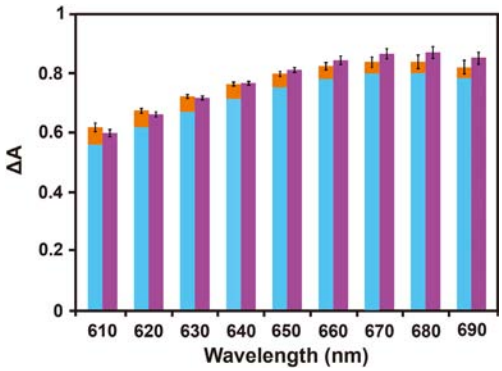

Fig. 2. Absorbance change (ΔA) of nonionic fluorosurfactant-capped gold nanoparticles from 610 nm to 690 nm in the presence of 4.0 μM Cys (blue), 0.5 μM Hcy (orange), or the mixture of 4.0 μM Cys and 0.5 μM Hcy (purple), respectively. The nonionic fluorosurfactant-capped gold colloidal solution at pH 6.5 contained 100 mM NaCl with 30 s incubation period at 50 °C, and the absorption values were measured at 30 s after addition of 4.0 μM Cys, 0.5 μM Hcy, and the mixture of 4.0 μM Cys and 0.5 μM Hcy. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Table 1
Average molar absorptivity for Cys and Hcy.

Wavelength (nm)	Aminothiols	Molar absorptivity[a] (10^6 L mol^{-1} cm^{-1})
630	Cys	0.159 ± 0.001
	Hcy	0.215 ± 0.002
650	Cys	0.166 ± 0.002
	Hcy	0.216 ± 0.003

[a] Mean ± SD of seven different concentrations of Cys, and nine different concentrations of Hcy.

3.3. Interfering effects from various sample matrixes

To assess the selectivity of the developed method, the effects of typical, common interferences present in human urine and plasma samples were investigated. A sample solution containing a fixed amount of Cys/Hcy and different concentrations of the interference compounds under evaluation was analyzed by the present method. A compound was considered as non-interfering if the analytical signal variation was ±5% when compared to the analytical signal obtained in the absence of the referred compound. The results revealed that all cationic ions did not interfere. Moreover, glucose, maltose, sucrose, sorbitol, fructose, thiourea and urea had no influence on the determination of Cys/Hcy. Therefore, it can be concluded that the proposed method shows very high selectivity for the determination of Cys and Hcy in biological samples. Note that the same selectivity was obtained for fluorosurfactant-functionalized silver nanoparticles.

3.4. Assay of Cys and Hcy in real samples

Under the optimum experimental conditions employed in the present study, the calibration curve was found to be linear from 0.5 to 4.5 μM for Cys/Hcy (Fig. 3). The limit of detection (LOD) for Cys/Hcy (S/N = 3) was 0.4 μM. In order to evaluate the applicability and reliability of the proposed methodology, it is applied for the

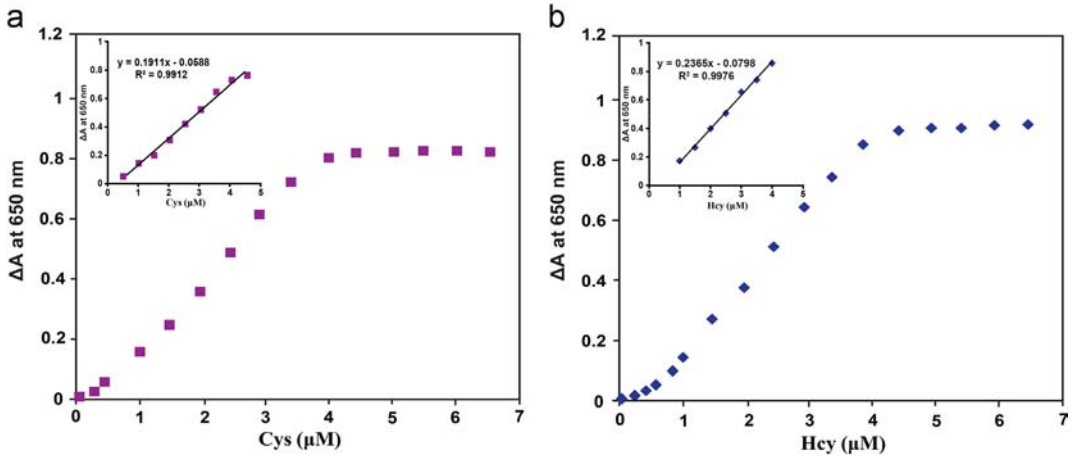

Fig. 3. Absorbance changes at 650 nm of nonionic fluorosurfactant-capped gold nanoparticles versus standard Cys or Hcy solution. The linear relationship of nonionic fluorosurfactant-capped gold nanoparticles versus Cys and Hcy. The linear range for Cys was 0.5–4.5 μM, and for Hcy was 1.0–4.0 μM. The nonionic fluorosurfactant-capped gold colloidal solution at pH 6.5 contained 100 mM NaCl with 30 s incubation period at 50 °C, and the absorption values were measured at 30 s after addition of Cys and Hcy.

determination of Cys and Hcy in human urine and plasma samples. These samples from some volunteers are collected from China–Japan Friendship Hospital (Beijing, China). After the sample reduction and appropriate dilution (ensuring that the signal fall within the linear range shown in Fig. 3), the concentrations in human urine and plasma samples are measured using a standard addition method and the results are shown in Table 2, which are in good agreement with those obtained in previous studies [20,22]. The accuracy of our method is tested by determining the recovery of known amounts of Cys and Hcy added to the real samples. The good recovery values from 97.2% to 106.7% indicate good accuracy of the proposed method. These results demonstrate that the novel approach is reliable and applicable.

3.5. *Generality of the additivity of individual surface plasmon resonance*

Demonstrating the generality of additivity of individual surface plasmon resonance requires employing other metal nanoparticles. Silver nanoparticles possess much higher extinction coefficients compared to gold nanoparticles of the same size, allowing sensitive colorimetric detection with minimal material consumption and higher sensitivity [23]. Furthermore, silver nanoparticles are biocompatible, non-toxic and of low cost [24–26]. Aminothiols induced aggregation of silver nanoparticles has been investigated extensively [27–29]. However, owing to the mutual interference of aminothiols, few studies have been reported for the selective detection of Cys or Hcy [30]. Herein, water-soluble nonionic fluorosurfactant-functionalized silver nanoparticles (8.0 nm) are also synthesized (Figs. S5 and S6). Similar to that seen in the gold nanoparticles [18], the aggregation of the silver nanoparticles could be linearly induced by either Cys or Hcy, resulting in an absorption decrease of the silver colloidal solution at 398 nm and an absorption increase at longer wavelengths (450–600 nm; Figs. S7 and S8); however, the silver nanoparticles do not respond to other amino acids (Fig. 4). Therefore, in order to further realize the generality of noble metal nanoparticle SPR additivity, fluorosurfactant-functionalized silver nanoparticles are also employed for the identification of Cys and Hcy in human urine and plasma samples. As shown in Table 3, the results are in agreement with those obtained by fluorosurfactant-functionalized gold nanoparticles (see Table 2). The experimental results indicate that absorbance additivity can be used for all noble metal nanoparticles to detect a variety of analytes capable of inducing nanoparticle aggregation.

Table 2
Assay of results for Cys and Hcy in human plasma and urine samples using nonionic fluorosurfactant-capped gold nanoparticles.

Sample	Biothiols	Determined biothiols (μM)	Spiked (μM)	Measured[a] (μM)	Recovery[a] (%)
Plasma 1	Cys	123.4 ± 4.1	150	149.5 ± 9.3	99.6 ± 6.2
			300	303.7 ± 12.1	101.3 ± 4.1
	Hcy	14.2 ± 2.9	10	10.2 ± 1.1	102.2 ± 11.4
			20	19.4 ± 1.3	97.2 ± 6.3
Plasma 2	Cys	117.2 ± 3.5	100	99.2 ± 7.3	99.2 ± 7.3
			200	200.2 ± 14.2	100.2 ± 7.1
	Hcy	8.9 ± 3.4	10	9.8 ± 1.1	98.6 ± 10.6
			20	20.8 ± 1.8	103.9 ± 4.6
Urine 1	Cys	173.1 ± 5.3	100	106.1 ± 8.1	106.1 ± 8.1
			200	213.4 ± 15.6	106.7 ± 7.8
	Hcy	6.2 ± 0.3	5	5.1 ± 0.4	102.9 ± 2.3
			15	14.8 ± 0.9	99.0 ± 5.8
Urine 2	Cys	103.9 ± 3.7	100	105.1 ± 6.4	105.1 ± 6.4
			200	197.2 ± 9.5	98.6 ± 4.8
	Hcy	7.9 ± 1.7	10	10.4 ± 1.8	103.5 ± 8.9
			15	15.8 ± 0.6	105.7 ± 4.1

[a] Mean ± SD of three measurements.

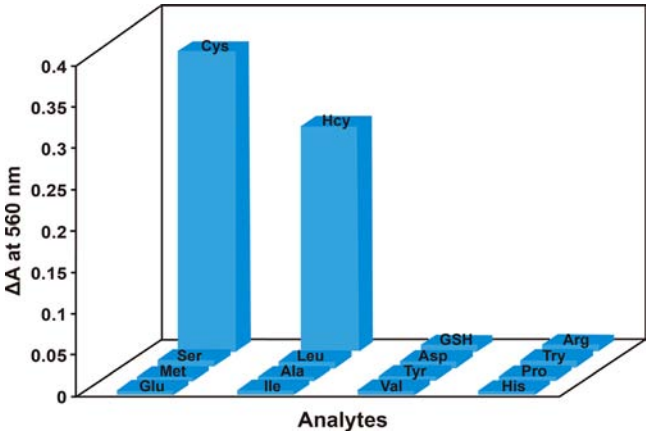

Fig. 4. Absorbance changes at 560 nm of nonionic fluorosurfactant-capped silver colloidal solution in the presence of various amino acids at pH 4.7. The concentration of each amino acid was 5.0 μM, and the absorbance changes were measured at 1.0 min after the addition of each amino acid. The nonionic fluorosurfactant-capped silver colloidal solution contained 70 mM NaCl at pH 4.7.

Table 3
Assay of results for Cys and Hcy in human plasma and urine samples using nonionic fluorosurfactant-capped silver nanoparticles.

Sample	Biothiols	Determined biothiols (μM)	Spiked (μM)	Measured[a] (μM)	Recovery[a] (%)
Plasma 1	Cys	140.6 ± 6.6	200	218.9 ± 11.2	109.4 ± 5.6
			300	289.1 ± 12.1	96.4 ± 4.1
	Hcy	4.7 ± 0.8	4	4.3 ± 0.3	107.1 ± 6.9
			8	8.6 ± 0.5	106.9 ± 5.8
Plasma 2	Cys	175.4 ± 5.5	200	189.7 ± 11.1	94.8 ± 5.5
			400	423.5 ± 5.3	105.9 ± 1.3
	Hcy	11.8 ± 0.5	10	10.2 ± 0.1	102.3 ± 1.4
			30	31.4 ± 2.2	104.7 ± 7.2
Urine 1	Cys	121.1 ± 7.9	200	197.3 ± 6.9	98.9 ± 3.5
			400	435.2 ± 2.1	108.8 ± 0.5
	Hcy	8.1 ± 0.1	2	2.1 ± 0.2	106.6 ± 6.9
			6	5.9 ± 0.6	98.2 ± 5.3
Urine 2	Cys	82.9 ± 5.6	100	94.5 ± 4.5	94.5 ± 4.5
			200	213.8 ± 6.1	106.9 ± 3.1
	Hcy	2.8 ± 0.5	2	1.9 ± 0.3	95.4 ± 5.9
			4	3.9 ± 0.1	98.4 ± 3.3

[a] Mean ± SD of three measurements.

4. Conclusions and perspectives

In summary, we reported a highly selective colorimetric detection method for Cys and Hcy in human urine and plasma samples using additivity of individual SPR band of fluorosurfactant-functionalized noble metal nanoparticles. To the best of our knowledge, this is the first report on using the additivity of the noble metal colloidal SPR band to identify the multicomponent mixture. If the surface functionalized ligands of nanoparticles are tuned, the present method is suitable for other multicomponent mixture. Accordingly, we believe that this approach reported here will lead to the development of a novel nanoparticle sensing scheme in a variety of fields with the assistance of computer and mathematical models.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.talanta.2013.03.073.

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