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A novel glutathione-stabilized silver-gold nano-alloy/Cu^{2+} combination as a fluorescent switch probe for L-histidine

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Abstract

A new and environmental friendly approach for the preparation of glutathione-stabilized silver-gold nano-alloy (GSH-AgAuNAs) with highly fluorescence was proposed in this work. The as-prepared GSH-AgAuNAs was characterized with various methods and utilized to couple with copper ion (Cu^{2+}) to form a fluorescent switch probe (GSH-AgAuNAs/Cu^{2+} combination) for the detection of L-histidine. The fluorescence of GSH-AgAuNAs was first quenched with an appropriate added amount of Cu^{2+} solution. Then in the presence of L-histidine, the GSH-AgAuNAs/Cu^{2+} combination solution exhibited an obvious fluorescence enhancement due to the special interaction between the imidazole group of L-histidine and Cu^{2+}. The strong chelation between L-histidine and Cu^{2+} illustrated the feasibility and selectivity for constructing a switch-on probe for the detection of L-histidine over other amino acids. Different from other detection methods toward L-histidine based on fluorescent nanomaterial, our work promised high selectivity, simplicity and the avoiding of organic solvents. Upon the optimal conditions, the newly constructed GSH-AgAuNAs/Cu^{2+} fluorescent probe showed a satisfied linear range from 2 to 40 μM, with the detection limit of 1.19 μM. The practical use of the combination GSH-AgAuNAs/Cu^{2+} in real human serum samples was tested, illustrating an applicable prospect towards L-histidine.

Key words: Silver-gold nano-alloy; L-Histidine; Copper ion; Fluorescent probe

1 Introduction

L-histidine, with an imidazole functional group, is a crucial and genetically coded natural α-amino acid in humans as well as other mammals. It has drawn intense attention for its function in the area of biochemistry because it is of positive significance in the growth and repair of tissues.[1] Besides, it also occupies an important position in dominating the transmission of metal elements in biological systems due to the imidazole side chain, which acts as a common coordinating ligand in metallo-proteins.[2] Recently, many studies have revealed that the lack of L-histidine in plasma may result in impaired nutritional state in patients with chronic kidney disease and affect the human growth factor.[3,4] On the contrary, its overexpression is closely related with plenty of diseases such as AIDS,[5] Alzheimer's disease,[6] cancer[7] and metabolic disorders like histidinemia.[8,9] Serving as a neurotransmitter or neuromodulator, L-histidine could also present vital functions in the central nervous system of mammals. Hence, different kinds of methods are developed for the quantitative detection of L-histidine in human serum including chromatography,[10] capillary electrophoresis,[11] surface-enhanced Raman scattering (SERS),[12] mass spectrometry,[13] voltammetry[14] and fluorometry.[15-18] Alternatively, the fluorescent detecting method has an advantage in the quantitative analysis of compounds contained a suitable functional group compared to those methods mentioned above, which usually demand complicated procedures or expensive equipment.

With the development of nanotechnology, noble metallic nanomaterial-based assays have attracted a growing number of research interests.[19-23] However, these

fluorescent probes had several drawbacks inherently such as the usage of organic solvents, labeling procedure and purification process. Compared to the single metal component forms, multicomponent nanomaterial like silver-gold nano-alloy had the potential to be a proper analytical agent due to their larger surface area and modified flexibility of both silver and gold components.[24] Up to now, although there are some researches about the detection of L-histidine using copper ion and fluorescent probe [25,26], some drawbacks like the organic pollution and inconvenience still demand a more advanced probe. Additionally, its outstanding stability and remarkable fluorescence properties have also shown applicable prospect when compared with some traditional organic dyes.[27] In this paper, a new and environmental friendly approach for the preparation of glutathione-stabilized silver-gold nano-alloy (GSH-AgAuNAs) with favorable fluorescence was proposed for the first time and meanwhile it was successfully applied to perform a selective quantitative detection of L-histidine.

Herein, a new type of "off/on" fluorescent probe was built by coupling the GSH-AgAuNAs with copper ion (Cu^{2+}). To be detailed, Cu^{2+} is bound through noncovalent interactions to the GSH-AgAuNAs, which could quench its fluorescence. Then the added analyte (L-histidine in this paper) displaces the Cu^{2+} to be released into the solution for recovering its intense fluorescence. This methods does not require linking fluorophore with receptor, which eliminated complicated labeling, laborious purification and toxic organic solvents. It is acknowledged that copper is a metal ion to form stable complexes with L-histidine,[28] thus we describe our ongoing

efforts in developing a simple and highly selective detection of L-histidine employing the newly GSH-AgAuNAs/Cu^{2+} combination as a fluorescent switch probe. To construct such a sensing system, the new GSH-AgAuNAs with remarkable fluorescence was first prepared. The fluorescence of GSH-AgAuNAs was then quenched with the addition of Cu^{2+}, forming the GSH-AgAuNAs/Cu^{2+} combination. In the presence of histidine, Cu^{2+} was snatched form the GSH-AgAuNAs/Cu^{2+} combination, resulting in the fluorescence recovery of the GSH-AgAuNAs. Compared to previous fluorescent metallic nanomaterial-based methods, the major advantage of the proposed method is the remarkable fluorescence of the GSH-AgAuNAs, with an environmental-friendly synthesized process. This newly prepared nano-alloy, having a special architecture, could ensure a non-toxic, label-free and cost-effective detection of L-histidine.

The developed GSH-AgAuNAs/Cu^{2+} combination acts as a fluorescent indicator in "switch-on" mode and the interaction between Cu^{2+} and imidazole residue contained in L-histidine offers high selectivity for L-histidine over other amono acids. By monitoring the remarkable fluorescence changes, we could quantitatively determine the concentration of target L-histidine with satisfied sensitivity and selectivity in the sample.

2 Experimental

2.1 Reagents and materials

Chloroauric acid ($HAuCl_4 \cdot 4H_2O$) was obtained from Kaima Biochemical

Reagent Co., Ltd. (Tianjin, China). Silver nitrate ($AgNO_3$), copper sulfate ($CuSO_4•5H_2O$), L-glutathione (GSH) were purchased from Sangon Bioengineering Co., Ltd. (Shanghai, China). Phosphate buffered saline (PBS buffer, 10 mM, pH7.40, containing 0.9 % NaCl) was used for all experiments.

L-histidine (L-His), alanine (Ala), leucine (Leu), glycine (Gly), lysine (Lys), tyrosine (Tyr), glutamic acid (Glu), tryptophan (Trp), Threonine (Thr), valine (Val), aspartic acid (Asp), phenylalanine (Phe), arginine (Arg), serine (Ser), glutathione (GSH) and Cystine (Cys) were of analytical grade purity and used as received. Millipore Milli-Q water (18 ΩM cm) supplied by a Millipore Milli-Q water purification system (Bedford, MA. USA) was applied in whole process of the experiment.

2.2 Instrumentations

The fluorescent spectra were measured on an F-7000 fluorescence spectrophotometer (Hitachi, Japan) equipped with a 1 cm × 1 cm quartz cuvette. All absorption spectra were recorded on a Lambda-950 UV-Vis spectrophotometer (PerkinElmer, USA). Fourier transform infrared (FT-IR) spectra were obtained from Magna 750 spectrophotometer (Nicolet, USA). The transmission electron microscope (TEM) image was collected using a JEM-1400 (JEOL, Japan). Powder X-ray diffraction (XRD) patterns of the samples were collected with an X-ray diffractometer (D8 Advance, Germany) in the range of 25-85° (2θ). Scanning electron microscope (SEM) image was taken by JSM-6360LA (JEOL, Japan). The photographs were

captured with a digital camera (BenQ GH220, China). All pH measurements were made with a PHS-3CA precision acidity meter (Dapu, China).

2.3 Preparation of Glutathione-stabilized silver-gold nano-alloy (GSH-AgAuNAs)

The preparation of GSH-AgAuNAs were composed of several procedures as shown in scheme 1a. In the beginning, 2.5 mL of 100 mM $AgNO_3$ solution and 2.5 mL of 100 mM trisodium citrate were added into 39 mL of deionized water with mixing evenly. Then, 6 mL of freshly prepared 50 mM $NaBH_4$ was added into the aqueous solution aforementioned accompanied with vigorous stirring. After stirring overnight, the AgNPs could be obtained. Next, an amount of 307.3 mg of GSH in powder was introduced to the as-prepared AgNPs solution. Afterwards, through core etching, the GSH-AgNPs (light orange in color) were obtained by incubating for 48 h under continuous stirring in a 70 °C oil bath.

Next, in the preparation of GSH-AgAuNAs, 4 mL of the abovementioned GSH-AgNPs solution was added into 10.8 mL of deionized water under stirring evenly. Then 1.2 mL of 10 mM $HAuCl_4 \cdot 4H_2O$ was added drop wise into this aqueous solution (gradually change to be yellow color) under vigorous stirring at room temperature. Then the mixture was allowed to proceed for 20 min under vigorous stirring. After a process of galvanic exchange, the mixture was centrifuged at 15000 rpm for 20 min to obtain precipitates (AgCl) for further characterization. The resulting supernatant of the mixture were thereafter added with excessive methanol (the ratio between water and methanol is 1:4) and the mixed solution was then

centrifuged again at 15000 rpm for 30 min. The newly obtained precipitates were dispersed in appropriate amount of PBS solution at pH7.40 and stored at 4°C for further use.

2.4 Fluorescent switch assay for L-histidine using GSH-AgAuNAs/Cu^{2+} combination

The GSH-AgAuNAs/Cu^{2+} combination was prepared by mixing 100 μL of GSH-AgAuNAs solution in PBS buffer (pH7.40, 10 mM) and 300 μL of 10 μM Cu^{2+} in a centrifuged tube. For the switch "on" detection of L-histidine, various volume of 1 mM L-histidine solution was added to the GSH-AgAuNAs/Cu^{2+} combination to present different concentration of L-histidine in the sensing range. The final volume of the reaction system was adjusted to 1000 μL with PBS buffer. The mixture was incubated for 20 min at room temperature. Afterwards, the fluorescence spectra of the mixture solution were recorded with excitation wavelength of 390 nm with the slit set of 10 nm.

2.5 Analysis of L-histidine in human serum sample

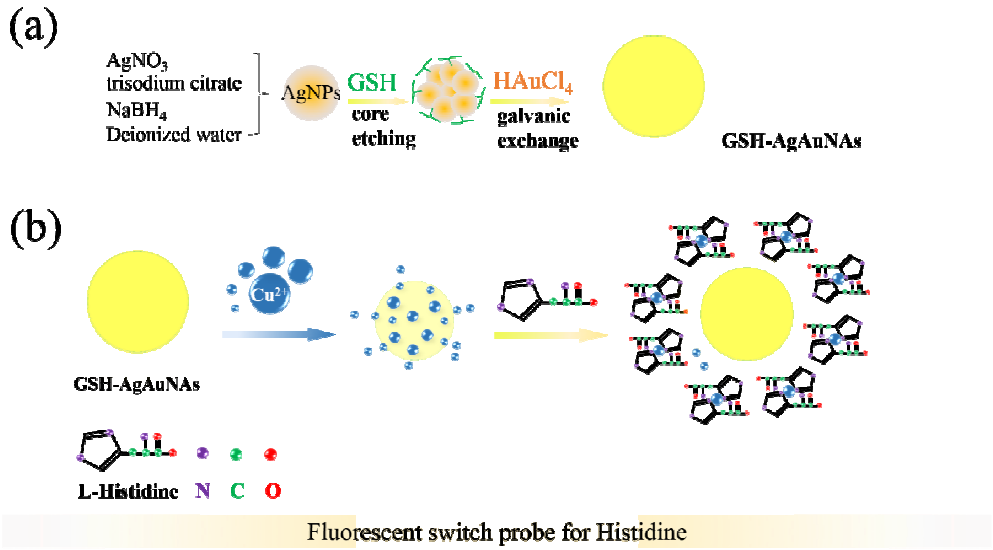
For a clearer description of the proposed fluorescent switch probe in practical application, a test was carried out using human serum samples (Obtained from infirmary of Shantou University). The original concentration of His in human serum samples were subjected to a 50-fold dilution with PBS buffer (10 mM, pH7.40). Different concentration of L-histidine was added into the diluted serum samples to prepare the spiked samples. The real sample measurements were made utilizing the

procedures mentioned above.

3 Results and discussion

3.1 Sensing mechanism of the proposed fluorescent switch probe

Scheme 1b illustrates the principle of the fluorescent switch probe toward target L-histidine. The fluorescence "switch on" strategy is based on the analyte competing for Cu^{2+}. The newly prepared GSH-AgAuNAs could perform a remarkable fluorescence in the reaction system. When Cu^{2+} was added afterwards, the fluorescence of the GSH-AgAuNAs was quenched sharply by Cu^{2+}. According to some reports, [29,30] it was possible that through fluorescence resonance energy transfer (FRET), Cu^{2+} could be a highly efficient fluorescent quencher due to its paramagnetic properties and quench the fluorescence of GSH-AgAuNAs via metal-metal interplay.[31]



Scheme 1 Schematic illustration of the proposed GSH-AgAuNAs/Cu^{2+} combination as a fluorescent switch probe L-histidine. (a) The preparation of GSH-AgAuNAs (b) The fluorescent

switch probe based on GSH-AgAuNAs/Cu^{2+} combination for the detection of L-histidine. *Schematic for demonstration only.

We know that the copper ion (Cu2+) have the empty d orbit and the ketonic oxygen in carboxyl of glutathione have lone pair electrons. As a result, the Cu2+ could coordinate with the ketonic oxygen. This property could make the Cu2+ combine with the surface of GSH-AgAuNAs and directly catch the electrons within the conduction band of the nano-alloy, which was excited by the light. Thus, the fluorescence of GSH-AgAuNas could be quenched by the copper ion.

When L-histidine was present in the complex system, Cu^{2+} could interact with the imidazole residue of L-histidine which was different from other amino acids. The strong chelation between Cu^{2+} and imidazole group of L-histidine leads to the liberation of Cu^{2+} from GSH-AgAuNAs. Subsequently the fluorescence of the released probe was extremely enhanced, which could be used as a selective "switch on" signal for histidine assay. Namely, our proposed GSH-AgAuNAs/Cu^{2+} combination could be used as a fluorescent switch probe in the quantitative detection of L-histidine.

3.2 Characterization of GSH-AgAuNAs

Owing to the quantum confinement and the discrete energy levels, strong fluorescence is considered an important property of our presented GSH-AgAuNAs. As shown in Fig. 1A, three dimensional fluorescence spectrum of GSH-AgAuNAs was recorded to present a more comprehensive profile. The GSH-AgAuNAs exhibited an emission spectrum with excitation and emission maxima at 390 nm and 578 nm.

The inset of Fig. 1A reveals that the GSH-AgAuNAs solution was light yellow in color under visible light (a), while it emitted a bright orange-red color under UV light at 365 nm (b).

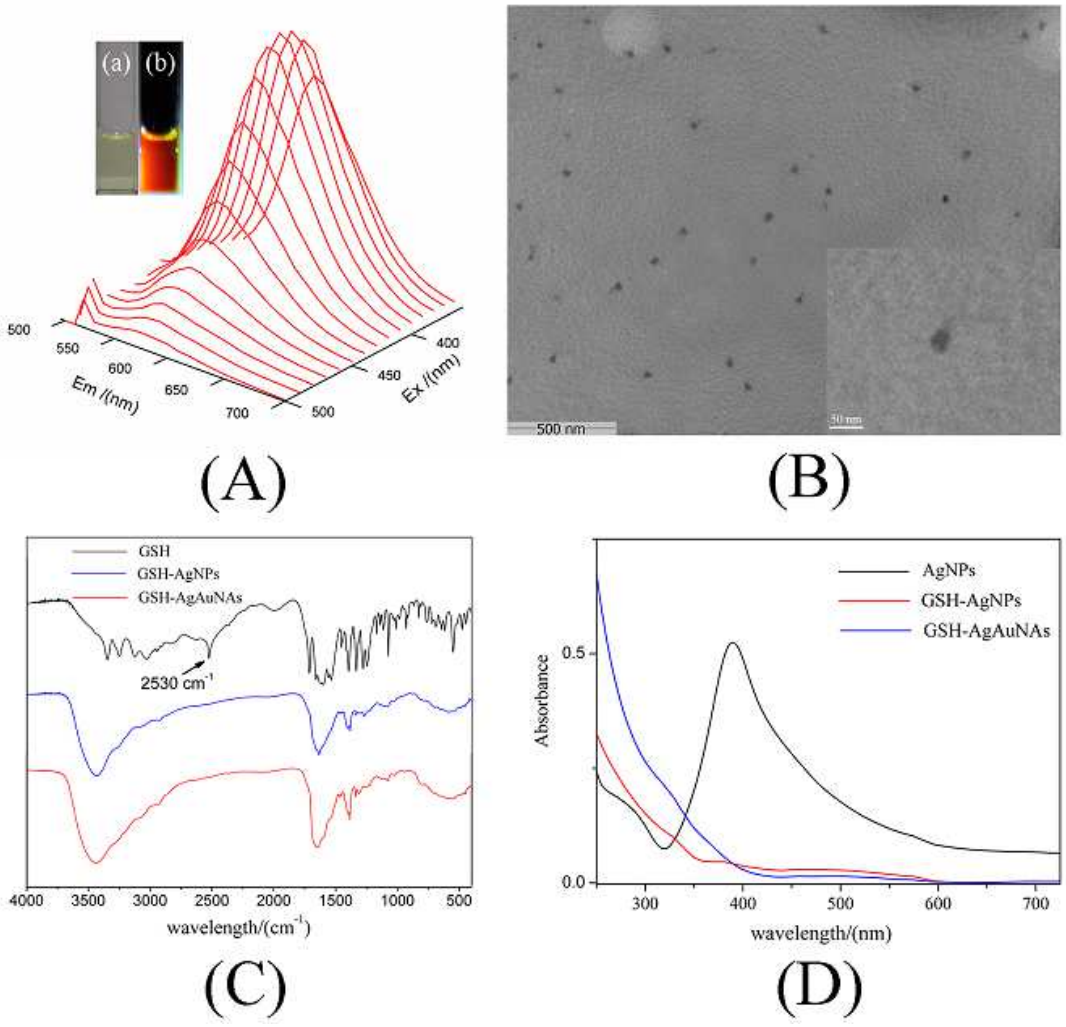


Fig. 1 The characterization of as-prepared GSH-AgAuNAs. (A) Three dimensional fluorescence spectrum of GSH-AgAuNAs. Inset: photographs of GSH-AgAuNAs under visible light (a) and UV light at 365 nm (b). (B) TEM image of GSH-AgAuNAs. (C) FT-IR spectra of GSH, GSH-AgNPs and GSH-AgAuNAs. (D) UV-Vis absorption spectra of AgNPs, GSH-AgNPs and GSH-AgAuNAs. All experiments were carried out under pH7.40 with PBS buffer (10 mM).

TEM image of GSH-AgAuNAs (Fig. 1B) demonstrated that the as prepared nano-alloy appeared to be spherical and dispersible, having an average diameter of 28.5 nm (The scale bar is 50 nm). Besides, the FT-IR spectra of GSH, GSH-AgNPs and GSH-AgAuNAs are recorded in Fig. 1C. It could be easily distinguished that the peak at about 2530 cm^{-1} is the characteristic peak of GSH (S-H stretching band). The disappearance of that band was also observed in both the GSH-AgNPs and GSH-AgAuNAs spectra, demonstrating that GSH was successfully used to stabilize the nano-alloy through Ag-S and Au-S interactions during the preparation of GSH-AgAuNAs.

Moreover, Fig. 1D has illustrated the absorption features of AgNPs, GSH-AgNPs and GSH-AgAuNAs. The surface plasmon resonance peak at 391 nm proved to the successful formation of AgNPs (black line) during the process of preparing the GSH-AgAuNAs. Both GSH-AgNPs and GSH-AgAuNAs exhibited strong and broad absorption features, which was similar to each other.

The adding amount of $HAuCl_4$ was optimized because it played an important part in the synthesis of GSH-AgAuNAs. As indicated in Fig. S1, with the increasing addition volume of $HAuCl_4$ (10 mM), the the corresponding emission intensity gradually increased with increasing volume of $HAuCl_4$ up to 1.2 mL. After that, the corresponding emission intensity gradually decreased. An excess amount of $HAuCl_4$ led to fluorescence quenching, probably due to it could damage the formation of metal alloy. As a result of it, 1.2 mL of 10 mM $HAuCl_4$ was chosen to be optimal conditions for the preparation of GSH-AgAuNAs.

Furthermore, Energy dispersive X-ray spectroscopy (EDS) spectrum of GSH-AgAuNAs was also tested to give a clear insight of the newly prepared nano-alloy. The EDS spectrum revealed that the GSH-AgAuNAs contained rather high level of silver and gold (12.63% and 23.52% respectively), which inferred that this nano-alloy were successfully prepared to some extent. We also tested the stability of the proposed GSH-AgAuNAs. According to our record, no obvious fluorescence change even after 6 week's storage in the dark at 4°C. All characteristic results indicated that the as-prepared nano-alloy could be a proper fluorescent agent.

As a complementary demonstration, XRD pattern and SEM image of AgCl were examined as shown in Fig. S2. AgCl is a byproduct during the preparation of GSH-AgAuNAs when $HAuCl_4$ was added. The XRD pattern of AgCl tested in this work was in accordance with the pattern reported in previous report.[32, 33] Combining the SEM image and the XRD pattern of AgCl, it proved the presence of AgCl.

3.3 Analytical performance of the proposed fluorescent switch probe for L-histidine

To demonstrate the feasibility of the proposed strategy, Cu^{2+} was initially added into the prepared GSH-AgAuNAs to test the quenching efficiency as shown in Fig. 2A. An optimum concentration of Cu^{2+} is an essential prerequisite for the "off/on" response to L-histidine because a superfluous or insufficient amount of Cu^{2+} would result in a lower detection limit and narrow detection respectively. As shown in Fig. 2A, with the addition of Cu^{2+} into GSH-AgAuNAs solution (in 10 mM PBS buffer, pH7.40), the instinct fluorescence of GSH-AgAuNAs (at 578 nm) was gradually

quenched. The fluorescence intensity dropped sharply and then reached a platform. As a result, 10 μM Cu^{2+} solution was used for the following experiments. Besides, in Fig. S3 (in the supplementary information), we presented the interaction time effect from the time scale of 5 min to 30 min. As we could see form the figure, the fluorescence intensity was at the maximum when we used 20 min as the interaction time. So 20 min interaction time was chosen as the best value.

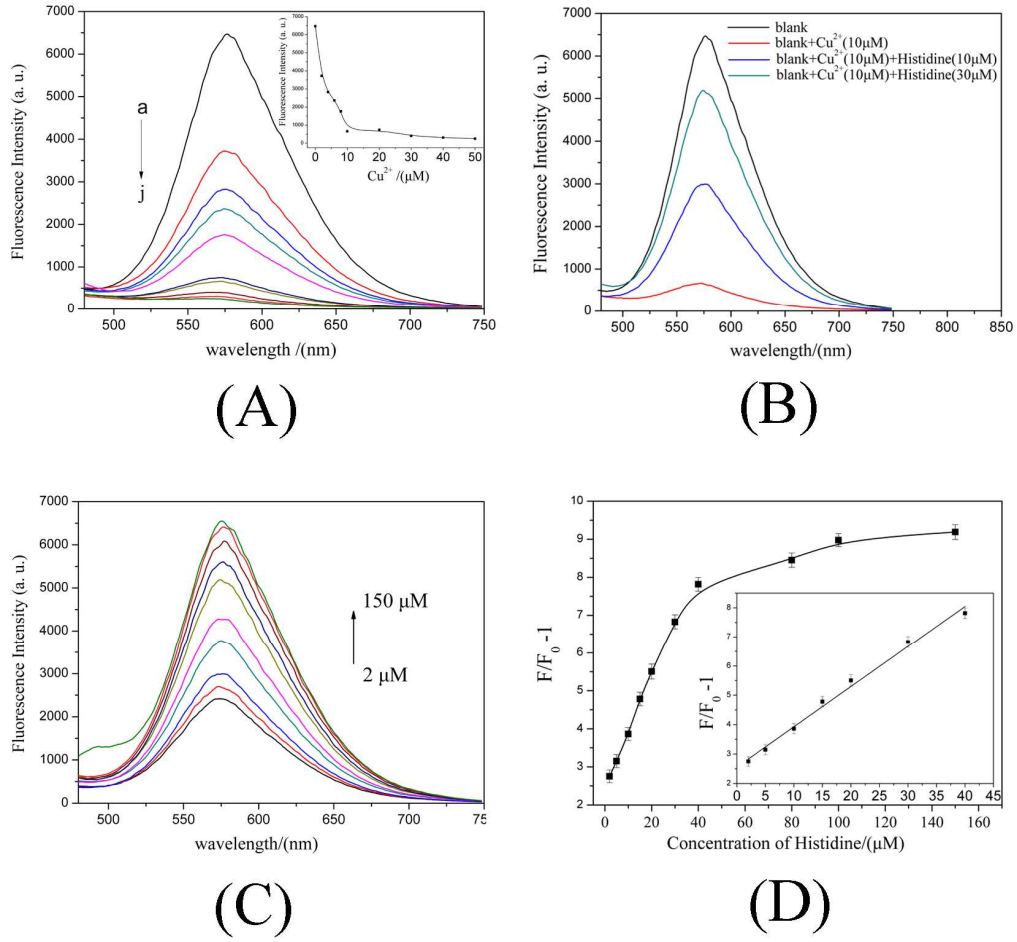


Fig. 2 The analytical performance of the proposed fluorescent switch probe. (A) Fluorescence response of GSH-AgAuNAs to different concentrations of Cu^{2+}. a-j: an increasing adding amount of Cu^{2+}. (B) The feasibility test of our proposed fluorescent switch probe with the addition of different amount of L-histidine. (C) Fluorescence response of the

GSH-AgAuNAs/Cu^{2+} combination to the increasing amount of L-histidine (2, 5, 10, 15, 20, 30, 40, 80, 100, 150 μM). (D) Plot of fluorescence responses of the GSH-AgAuNAs/Cu^{2+} combination solution to various concentrations of L-histidine; the inset shows the linear range of the calibration curve. All experiments were carried out under pH7.40 with PBS buffer (10 mM).

When L-histidine were added into the mixture of GSH-AgAuNAs/Cu^{2+} combination as indicated in Fig. 2B, an obvious fluorescence enhancement could be observed (blue and green line). These observations demonstrated the feasibility of this method for determination of L-histidine with our fluorescent signal "switch-on" proposal.

To investigate the performance of our strategy for L-histidine detection, different concentrations of L-histidine were added into the GSH-AgAuNAs/Cu^{2+} combination solution. As seen in Fig. 2C, the fluorescence intensity gradually restored with the increasing concentration of L-histidine, indicating that the binding ability between Cu^{2+} and the imidazole moiety on L-histidine side chain was stronger than the coordination interaction between Cu^{2+} and GSH-AgAuNAs.

As presented in Fig. 2D, the characteristics of the calibration curve and detection limit of the fluorescent probe were investigated. It could be seen that the fluorescent enhancement constant (F/F_0 -1) is sensitive to the concentration of L-histidine (F and F_0 represented respectively the fluorescence intensity of GSH-AgAuNAs/Cu^{2+} combination solution after and before the L-histidine was added into the reaction mixture). From the inset of Fig. 2D, a linear relationship was observed over the range of 2-40 μM (R^2=0.9914) with a detection limit of 1.19 μM.[34] The relative standard

deviation (RSD) for 5 detections of 15 μM L-histidine was 2.58%, indicating a good repeatability of measurement. For comparison, the detection limits and linear ranges for L-histidine detection by different methods were summarized in Table S1 in the supplementary information. As seen in the Table S1, although the first two methods had better detection limit than our presented assay, our work had a wider linear range than those two methods. At this point, in order to get the most experimental result, the linear range and detection limit of our presented work would be the most appropriate for the detection of histidine because of its applied values.

3.4 Selectivity and practical application

Selectivity is an essential element for our proposed fluorescent switch probe. As illustrated in Fig. 3, the effects of other amino acids or potential coexisting biomolecules on the response from the GSH-AgAuNAs/Cu^{2+} combination are tested.

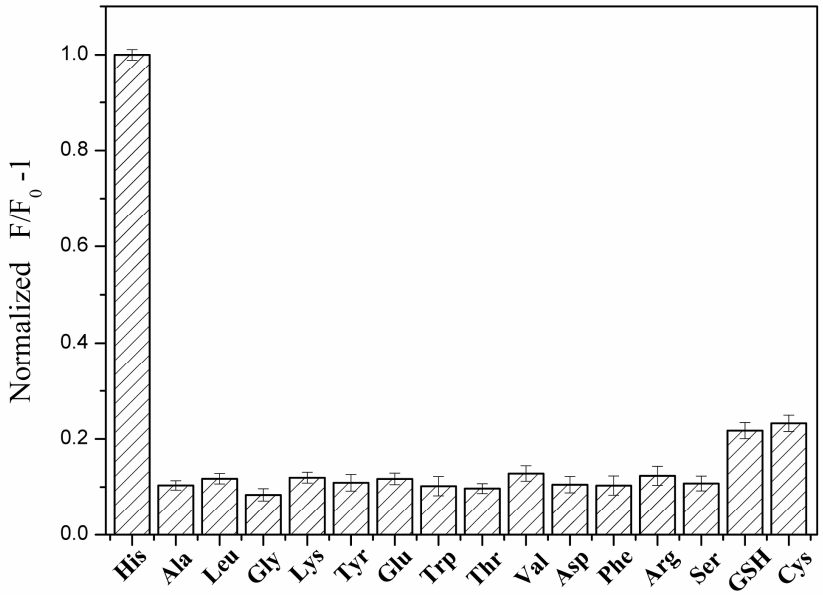


Fig. 3 Fluorescence response of the GSH-AgAuNAs/Cu^{2+} probe to histidine and various amino acids. The Table 1 (inset) indicated the testing results of different spiked L-histidine concentration in human serum. Conditions: pH7.40 in PBS buffer (10 mM).

No obvious signals from these tested amino acids or biomolecules are observed, demonstrating good selectivity of the proposed method for L-histidine (His). This mainly attributed to the imidazole side chain moiety, which was the most distinct structural difference between L-histidine and other amino acids. Hence, the proposed method is selective for the determination of L-histidine in a mixture of amino acids.

In order to demonstrate the practical application of our fluorescent probe in human serum, 50-fold diluted human serum with 10 mM PBS buffer solution. The results were summarized in Table 1.

Table 1 Determination results of Histidine in human serum [a]

Sample	Histidine added (μM)	Histidine detected (μM)	Recovery (%)	RSD (%, n=3)
1	3.00	3.15	105.0	3.67
2	6.00	6.23	103.8	1.47
3	12.00	12.50	104.2	1.31
4	25.00	25.61	102.4	1.33
5	35.00	36.20	103.4	1.57

[a] The original concentration of L-histidine in human serum samples were subjected to a 50-fold dilution with PBS. Each data was given as the average value obtained from three independent experiments.

With different amount of L-histidine added into the human serum medium, the detected concentration could be obtained through the measurement of the fluorescence intensity of GSH-AgAuNAs/Cu^{2+} system. The recovery values were in

the range from 102.4% to 105.0% under optimal condition, indicating the designed fluorescent nano-alloy based switch probe still worked well and had a reliable result in real human serum sample application.

4 Conclusions

To draw a conclusion, our work demonstrated a new and environmental friendly approach for the synthesis of silver-gold nano-alloy using glutathione as a stabilized agent. Besides, by coupling with Cu^{2+}, a new glutathione stabilized silver-gold nano-alloy/Cu^{2+} combination as a fluorescent switch probe was constructed for the detection of L-histidine. With the use of this combination, the proposed approach exhibited a linear range of 2-40 μM towards L-histidine with a detection limit of 1.19 μM. In addition, our proposal has been already utilized to detect target L-histidine in real human serum sample and achieved a satisfied result. Compared with other fluorescent agents based assay for L-histidine, our presented method had the potential to be an applicable sensing strategy for L-histidine due to its favorable selectivity. More inspiringly, our proposed fluorescent switch probe does not require sophisticated instruments and it could provide a green and convenient assay for the target L-histidine.

Acknowledgements

References

1 R. M. Kong, X. B. Zhang, Z. Chen, H. M. Meng, Z. L. Song, W. Tan, G. L. Shen and R. Q. Yu, *Anal. Chem*., 2011, **83**, 7603-7607.

2 X. Li, H. Ma, L. Nie, M. Sun and S. Xiong, *Anal. Chim. Acta*., 2004, **515**, 255-260.

3 J. D. Kopple and M. E. Swendseid, *J. Clin. Invest*., 1975, **55**, 881-891.

4 Y. Kusakari, S. Nishikawa, S. I. Ishiguro and M. Tamai, *Curr. Eye Res*., 1997, **16**, 600-604.

5 A. L. Jones, M. D. Hulett, C. R. Parish, *Immunol. Cell Biol*., 2005, **83**, 106-118.

6 S. Seshadri, A. Beiser, J. Selhub, P. F. Jacques, I. H. Rosenberg, R. B. D'Agostino, P. W. Wilson, P. A. Wolf, *N. Engl. J. Med*., 2002, **346**, 476-483.

7 C. Verri, L. Roz, D. Conte, T. Liloglou, A. Livio, A. Vesin, A. Fabbri, F. Andriani, C. Brambilla, L. Tavecchio, G. Calarco, E. Calabro, A. Mancini, D. Tosi, P. Bossi, J. K. Field, E. Braimbilla, G. Sozzi, *Am. J. Respir. Crit. Care Med*., 2009, **179**, 396-401.

8 P. M. Kovach and M. E. Meyerhoff, *Anal. Chem*., 1982, **54**, 217-220;

9 S. Liu, F. Shi, L. Chen and X. Su, *Analyst*, 2013, **138**, 5819-5825.

10 N. Tateda, K. Matsuhisa, K. Hasebe, N. Kitajima and T. Miura, *J. Chromatogr. B: Biomed. Sci. Appl*., 1998, **718**, 235-241.

11 J. Meng, W. Zhang, C. X. Cao, L. Y. Fan, J. Wu and Q. L. Wang, *Analyst*, 2010, **135**, 1592-1599.

12 S. Ye, Y. Guo and J. Xiao, *Chem. Commun*., 2013, **49**, 3643-3645.

13 T. Natsume, H. Nakayama, Ö. Jansson, T. Isobe, K.Takio, K. Mikoshiba, *Anal. Chem*., 2000, **72**, 4193-4198.

14 M. Shahlaei, M. B. Gholivand, A. Pourhossein, *Electroanalysis*, 2009, **21**, 2499-2502.

15 S. Y. Qiu, M. Miao, T. X. Wang, Z. Y. Lin, L. H. Guo, B. Qiuamd and G. N. Chen, *Biosens. Bioelectron*., 2013, **42**, 332-336.

16 X. Li, H. Ma, L. Nie, M. Sun and S. Xiong, *Anal. Chim. Acta*, 2004, **515**, 255-260.

17 Z. Huang, J. Du, J. Zhang, X. Q. Yu and L. Pu, *Chem. Commun*., 2012, **48**, 3412-3414.

18 X. Li, H. Ma, S. Dong, X. Duan and S. Liang, *Talanta*, 2004, **62**, 367-371.

19 I. Diez and R. H. A. Ras, *Nanoscale*, 2011, **3**, 1925-2344.

20 X. Zheng, T. Yao, Y. Zhu and S. Shi, *Biosens. Bioelectron*., 2015, **66**, 103-108.

21 H. C. Yeh, J. Sharma, J. J. Han, J. S. Martinez and J. H. Werner, *Nano Lett*., 2010, **10**, 3106-3110.

22 B. Han and E. Wang, *Biosens. Bioelectron*., 2011, **26**, 2585-3589.

23 Y. C. Shiang, C. C. Huang and H. T. Chang, *Chem. Commun*., 2009, **23**, 3437-3439.

24 S. Pande, S. K. Ghosh, S. Praharaj, S. Panigrahi, S. Basu, S. Jana, A. Pal, T. Tsukuda and T. Pal, *J. Phys. Chem. C*., 2007, **111**, 10806-10813.

25 Q. H. You, A. W. M. Lee, W. H. Chan, X. M. Zhu and K. C. F. Leung, *Chem. Comm.* 2014, **50**, 6207-6210.

26 J. Sun, F. Yang, D. Zhao, C. Chen and X. Yang, *Appl. Mater. Interfaces*, 2015, **7**, 6860-6866.

27 I. A. Larmour and D. Graham, *Analyst*, 2011, **136**, 3831-3853.

28 S. H. Seo, S. Kim and M. S. Han, *Anal. Methods*, 2014, **6**, 73-76.

29 X. R. He, H. F. Liu, Y. Li, S. Wang, N. Wang, J. C. Xiao, X. Xu and D. B. Zhu, *Adv. Mater*., 2005, **17**, 2811-2815.

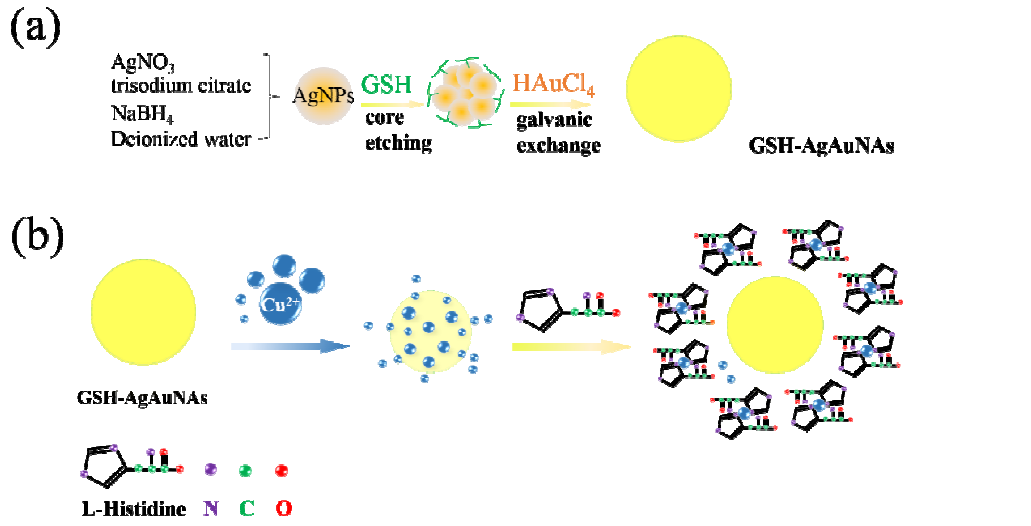
30 P. Yang, Y. Zhao, Y. Lu, Q. Z. Xu, X. W. Xu, L, Dong and S. H. Yu, *ACS Nano*, 2011, **5**, 2147-2154.

31 M. Zhang and B. C. Ye, *Analyst*, 2011, **136**, 5139-5142.

32 P. Wang, B. Huang, X. Qin, X. Zhang, Y. Dai, J. Wei and M. H. Whangbo, Angew. Chem. Int. Ed., 2008, **47**, 7931-7933.

33 J. Bai, Y. Li, S. Yang, J. Du, S. Wang, C. Zhang, Q. Yang and X. Chen, Nanotechnology, 2007, **18**, 305601-305605.

34 X. Huang, Y. Li, X. Huang, Y. Chen and W. Gao, *RSC Adv*., 2015, **5**, 30628-30724.



Fluorescent switch probe for Histidine

The synthesis of glutathione stabilized silver-gold nano-alloy and the detection mechanism of L-histidine using the fluorescent switch probe