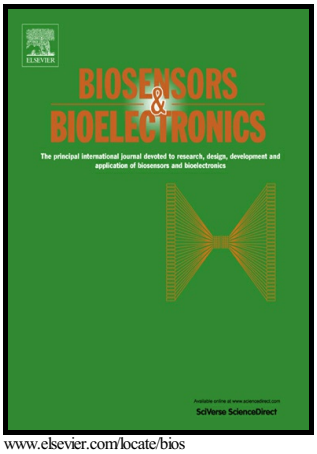

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Colorimetric Response of Peptide Modified Gold Nanoparticles: An Original Assay for Ultrasensitive Silver Detection

Xinyi Li, Zitong Wu, Xiaodong Zhou*, Jiming Hu*

Key Laboratory of Analytical Chemistry for Biology and Medicine (Ministry of Education), College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, P. R. China

zhouxd@whu.edu.cn (X. Zhou)

jmhu@whu.edu.cn (J. Hu).

*** Corresponding authors.**

Abstract

In this article, we for the first time present an original and ultrasensitive assay to detect Ag^+ ions, with which the aggregation of the given peptide-modified gold nanoparticles (peptide-AuNPs) can be triggered by the interaction between peptides and Ag^+. The approach has rarely been used in the colorimetric determination of Ag^+, because the mechanism of the above-mentioned interaction has not been studied through. In our assay, the principle of this interaction was investigated. Moreover, we applied it in the design of an extremely sensitive sensor for Ag^+ detection with great selectivity. It is the Ag^+-induced folding structure of the peptides that leads to the aggregation of peptide-AuNPs. The aggregation involves the formation of

4-coordinated complexes between Ag^+ and peptides via bonding with the carbonyl oxygen and the nitrogen of the α-amino group in the peptides. The result shows that Ag^+ ions can be selectively detected as low as 7.4 nM with a linear range of 10 to 1000 nM. Compared with other approaches, the proposed approach demonstrates superior simplicity, sensitivity, stability and time-saving. Furthermore, the biosensor excels in the practical application in water samples (e.g., lake, tap and drinking water) owing to its non-interference and on-site rapid determination.

Keywords: Colorimetric assay; Peptide-AuNPs; Ag^+ detection; Aggregation

1. Introduction

Silver ions (Ag^+) are one of the most toxic heavy metals, which may have adverse effects on the environment and bring about severe risks to human health. Bound with metabolites, they suppress sulfhydryl enzymes, and may lead to silver accumulation in the body and cause argyria and various disorders (**Ceresa et al., 2002; Huang et al., 2011; Ratte, 1999**). Because of their wide existence in water, soil and food, they are also harmful to plants and animals (**Li et al., 2009**). Therefore, the monitoring of Ag^+ levels in water (drinking, sea, lake, etc.) is crucial in terms of environmental analysis, toxicology, water safety and water quality. More attention is paid nowadays to monitor silver ions in aqueous solution, which greatly motivates the development

of new methods with high selectivity and sensitivity. The regular analysis methods for Ag^+ determination from water samples include atomic absorption (AAS) (**Bianco et al., 2015**), atomic emission (AES) (**Han et al., 2006**), inductively coupled plasma mass spectrometry (ICP-MS) (**Guo et al., 2011; Yuan et al., 2014**), electrochemical methods (**Gong and Li, 2011; Hourani, 1994; Miao et al., 2013; Sun et al., 2015**) and so on. In spite of their high selectivity and sensitivity, these methods suffer from being costly, labor-intensive, time-wasting, and non-portable. Recently, researches on phosphorescent transition-metal complexes functioning as biomolecular probes have gained popularity, which generally exhibit a large Stokes shift and provide a long-lived luminescence (**Lu et al., 2016; Tobita and Yoshihara, 2016; Zeng et al., 2016**). Leung's group has established a sensing platform to employ a G-quadruplex-selective iridium (III) complex for TDG activity detection (**Lin et al., 2016**). Ma's group has developed a novel luminescent iridium (III) complex incorporating an Al^{3+} receptor as metal ions chemosensor, especially suitable for complicated biological samples (**Wang et al., 2016**). These methods may pave us a new avenue for the expansion of distinct biosensor for metal ions. And yet, most of them expose such defects as complication, difficult synthesis of materials, unsatisfactory sensitivity and selectivity. Therefore, it is highly requested to develop simple, rapid, sensitive and portable systems for Ag^+ detection.

It is extremely attractive to use colorimetric approaches for their simplicity, cost effectiveness and rapidity. Moreover, the colorimetric response of these sensors can be easily read out with the naked eyes or using a UV-vis spectrophotometer

(**Anderson et al., 1996; Dagnall et al., 1962**). Particularly, colorimetric sensors of metal (mostly Au and Ag) nanoparticles (NPs) have attracted a great deal of attention due to its high extinction coefficient in the visible region and color-tunable behavior dependent on the interparticle distance (**Chai et al., 2010; Kew et al., 2006**). Recently, several groups have proposed colorimetric assays of Ag^+ via manipulating AuNPs with different recognition units, most of which performed the whole detection based on cytosine-Ag^+-cytosine coordination chemistry**,** such as aptamers, oligonucleotides (**Li et al., 2009; Ono et al., 2008**), and others (**He et al., 2016; Lin et al., 2010; Wang et al., 2011**). Although they exhibited good response to Ag^+, employment of DNA oligomers could limit the practical applications owing to their unsteady structure, painstaking procedures and low water solubility (**Mirkin et al., 1996; Torigoe et al., 2006; Zhang et al., 2010**). Hence, a novel functionalized AuNPs for simple, sensitive and steady test of Ag^+ is greatly desired. Accordingly, peptides have been found to be more advantageous over other ligands (e.g., DNA, amino acids, aptamers) for their easier modification and greater flexibility in sequences design as well as properties for easier control. And peptides with several specific sequences have been reported to stabilize the AuNPs due to their relatively lower isoelectric point (**Wu et al., 2015**). Nevertheless, few reports on Ag^+ detection based on the interaction between Ag^+ and peptides have been published so far. As is well known, strong affinity of certain single amino acids (e.g., cysteine, arginine) toward Ag^+ has been reported (**Lee et al., 1998**), but practical testing and applications are blocked due to the bad selectivity of individual amino acid towards metal ions (**Lou et al., 2011;**

Sener et al., 2014; Zhang et al., 2012). Worthy of attention is that Ag^+ can be 4-coordinated with the carbonyl oxygen and the nitrogen of the α-amino group for the strong affinities, which can have great specificity for Ag^+ ions (**Forbes et al., 2007; Guo et al., 2010; Remko et al., 2008**). Based on these findings, we have designed the given peptide (RFPRGGDD), which can not only keep the AuNPs stable at its relatively lower isoelectric point but also easily 4-coordinate with Ag^+ to optimize the selectivity. But, in view of its complexity, the behavior between the given peptides and Ag^+ has not been clarified yet, so there are still some debates existing. Consequently, the mechanism of this system is worth further investigation.

In our assay, we develop an original system based on peptides stabilized AuNPs for rapid Ag^+ detection in water samples. These new behaviors between peptides and Ag^+ ions have rarely been studied before, and therefore, the mechanism of this interaction is also studied in our work, which can throw light upon the above indistinct principles. We simply use the unique behavior to promote the aggregation of peptide-AuNPs in the presence of Ag^+ owing to the Ag^+-induced folding structure of peptides. It generates a strong surface plasmon resonance (SPR) absorption (**Mulvaney., 1996**) and apparent color change from red to purple even gray, from which Ag^+ can be easily recognized and determined. We further demonstrate the analytical potential of this method for monitoring Ag^+ in water samples. Compared with several methods based on colorimetric detection of Ag^+, our approach provides a distinctive way to monitor Ag^+ in consideration of its simplicity, rapidity, superior sensitivity and selectivity (**see Supporting Information, Table S1**).

2. Experimental section

2.1. Materials

Tri-sodium citrate ($C_6H_5Na_3O_7$), ethylenediaminetetraacetic acid (EDTA), $HAuCl_4{\cdot}3H_2O$, LiCl, KCl, $MgCl_2$, $CaCl_2$, $Pb(NO_3)_2$, NaCl, $Fe(NO_3)_3{\cdot}9H_2O$, $Hg(NO_3){\cdot}2H_2O$, $CrCl_3$, $MnCl_2$, $FeCl_3$, $CoCl_2$, $NiCl_2$, $CuCl_2$, $ZnCl_2$, $AgNO_3$, KNO_3, K_2SO_4, NaOAc, $NaNO_2$, NaF, K_2CO_3 and $Cd(NO_3)_2{\cdot}4H_2O$ were purchased from Sinopharm Chemical Reagent (China). Peptide I, Arg-Phe-Pro-Arg-Gly-Gly-Asp-Asp (RFPRGGDD) and peptide II, Arg-Arg-Gly-Gly-Asp-Asp (RRGGDD) and peptide III Arg-Arg-Asp-Asp (RRDD) were 95% pure and obtained from Sangon Biotechnology Inc. (Shanghai, China). In our assay, phosphate buffer (PB) was prepared as the buffer. All solutions and the buffers in the experiments were obtained using ultrapure water.

2.2. Instrumentations

A Shimadzu UV-2550 was employed to obtain the absorption spectra of peptide-AuNPs before and after the addition of Ag^+. Transmission Electron Microscope (TEM) images were obtained with a JEM-2100 (HR) microscope at acceleration voltage at 200 kV. And X-ray photoelectron spectroscopy (XPS) spectra were collected in an ESCALAB 250Xi spectrometer and samples were vacuum dried before XPS measurements.

Mass spectra were obtained using a MALDI-TOF MS (AB SCIEX TOF/TOF 5800) instrument, using detection by reflection to generate the spectra. Samples were mixed

with a 2, 5-dihydroxybenzoic acid (DHB) matrix in a 1:1 ratio, and 2 μL of each mix was deposited on a micro scout simple-holder slide.

2.3. Synthesis of AuNPs and peptide-AuNPs

According to published procedures, AuNPs with a diameter of ~30 nm were synthesized by reduction of the $HAuCl_4$ salt via using sodium citrate (**Enu□stu□n and Turkevich, 1963**). In brief, 100 mL of 0.01% (w/w) $HAuCl_4$ was heated by boiling under vigorous magnetic stirring. Then, 1.5 mL of 33.8 mM sodium citrate solution was added promptly. The mixture was kept boiling and magnetic stirring for 20 min after turning red, then cooled to room temperature. Finally, AuNPs was obtained and the volume of the solution was regulated to 100 mL.

Briefly, peptide I was dissolved in PB (10 mM, pH 7.4). Then, 100 μL of peptide I (1 mM) was added to AuNPs (2 mL).The reaction mixture was incubated at room temperature for several minutes. The free peptide molecules in solution were removed by centrifugation at 6000 rpm for 10 min, finally the peptide-AuNPs were obtained.

2.4. Colorimetric detection of Ag^+

$AgNO_3$ stock solution was prepared for the Ag^+ assay. Diverse concentrations of Ag^+ (0.1-500 μM) were obtained by serially diluting the stock solution to test the sensitivity limits of peptide-AuNPs. Briefly, 90 μL of peptide-AuNPs solution was added 5 μL of Ag^+ with different concentrations. Otherwise, we added 5 μL EDTA (0.1 mM) into the solution to mask Hg^{2+} for Ag^+ sensing, resulting in a better

selectivity. Therefore, the final volume of the reaction mixture was regulated to 100 μL. The peptide-AuNPs turned purple as they met Ag^{+}, indicating formation of aggregates.

The color changes were detected by UV−vis spectrophotometer. The absorption measurements between 400 nm and 850 nm were implemented. The absorption values of the solution at 550 nm and 700 nm corresponds to the quantities of dispersed and aggregated AuNPs, respectively. Therefore, the state of dispersed to aggregated AuNPs can be presented by the ratio of the absorption value at 700 nm to that at 550 nm ($A_{700\ nm}/A_{550\ nm}$). It is clear that the addition of Ag^{+} to a solution results in an increasing value of $A_{700\ nm}/A_{550\ nm}$. All experiments were repeated three times, respectively.

2.5. Selectivity and recovery test for Ag^{+}

In the experiments for selectivity and practical assay, all samples were tested in the above-mentioned conditions. We explored the selectivity of our proposed strategy for Ag^{+} over other potential common ions (Hg^{2+}, Cr^{3+}, Mn^{2+}, Zn^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Li^{+}, K^{+}, Na^{+}, SO_4^{2-}, AcO^{-}, NO_2^{-}, F^{-}, CO_3^{2-}, Cl^{-}, NO_3^{-}). Besides that, the influence of these anions on Ag^{+} binding to peptide-AuNPs was also investigated. UV-vis spectra showed that the method exhibited great selectivity.

The recovery experiments were accomplished using Ag^{+}-spiked lake water, tap water and drinking water. Compared with the standard curve of Ag^{+}, we confirmed the Ag^{+} concentration in the samples by using the response ($A_{700\ nm}/A_{550\ nm}$) of the

colorimetric assay against Ag^+-spiked water samples. In consequence, recovery values were calculated.

3. Results and discussion

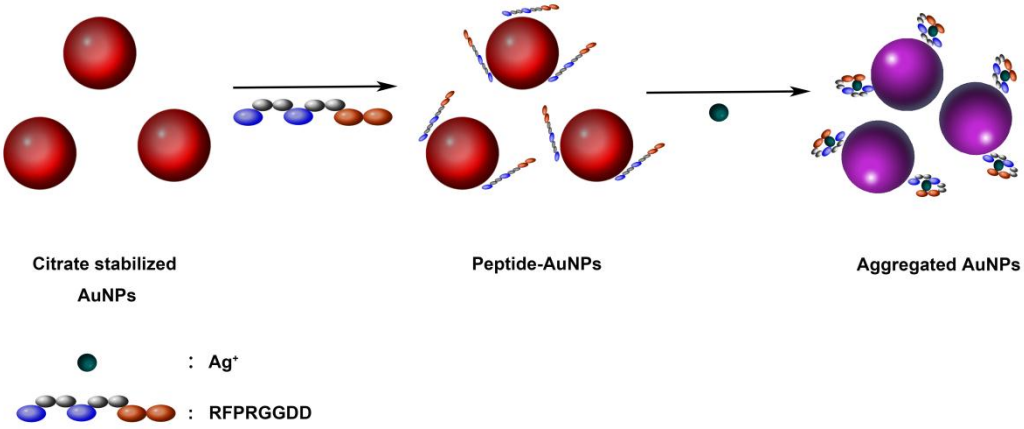
3.1. Evidences of Ag^+-induced aggregation of peptide-AuNPs

The peptide I (RFPRGGDD) can be bound to the surface of AuNPs through Au-N bonds owing to the available -NH_2 at the N-terminal of peptides. The XPS spectra showed characteristic N1s peak at 400 eV after the addition of peptide to AuNPs, which was in line with the binding of peptides (**Fig. S1**). Theoretically, the peptide-AuNPs exhibit much better stability than bare AuNPs because the peptide I is negatively charged at pH 7.4 to form a uniformly charged layer, increasing the electrostatic repulsion between AuNPs. Additionally, peptide I has two free -COOH groups and two free -NH_2 groups in the side chain owing to the aspartic acids and arginines, which could form the 4-coordinated complex with Ag^+ to further induce the aggregation of AuNPs. As shown in **Fig. S1C**, characteristic N1s peak and Ag3d peak were observed, it was validated that peptides were still on the surface of AuNPs after coordination with Ag^+. Thus, there are two possible explanations about the mechanism of Ag^+-induced aggregation of peptide-AuNPs. The first explanation is that Ag^+ 4-coordinates with -COOH groups and -NH_2 groups of two peptides in different AuNPs, which forms a bridge to reduce the distance between AuNPs and then causes the aggregation (are shown in **Scheme S1A**). The second interpretation is that Ag^+ four-coordinates with -COOH groups and -NH_2 groups of one peptide to

change the structure of this peptide, reducing the electrostatic repulsion and steric hindrance between peptide-AuNPs (are shown in **Scheme S1B**). Therefore, MALDI-TOF MS was employed to prove the principle.

MALDI-TOF MS spectra proved that the peptide I could bind with Ag^+ (**Fig. S2**), as we expected. In the spectrum (**Fig. S2a**), m/z = 919.59 was observed. While Ag^+ bound with peptide I, there was no doubt that a calculated molecular weight of 1027.59 could be surveyed. In the spectrum (**Fig. S2b**), m/z = 1027.34 was observed, which proved the existence of Ag^+-peptide I (Ag^+-RFPRGGDD) complexes. Then, similarily we confirmed the existence of Ag^+-peptide III (Ag^+-RRDD) complexes since m/z = 669.35 was observed (**Fig. S2d**). Comparing the two results, we can find that little adverse impact on the combining capacity with Ag+ is produced by adding several neutral amino acids into the sequence RRDD. Based on these foundations, we obtained peptide II (RRGGDD) by adding some appropriate neutral amino acid into peptide III (RRDD). Further, we used the two peptides to prove the mechanism. The mixture of peptide II and peptide III was treated with Ag^+ (4 mM) at room temperature, and then detected by MALDI-TOF MS. If the mechanism was the first explanation as mentioned above, m/z = 725.84 could be viewed in the spectrum. As shown in **Fig. S3**, m/z = 725.84 was not observed, which directly vetoed the first explanation. In other words, the structure of two peptides in different AuNPs coordinating with Ag^+ ions did not exist. It was also found that RRGGDD had an advantage over RRDD in bonding with Ag^+. In consequence, MALDI-TOF MS experiments revealed that the Ag^+-induced folding structure of peptides was the

mechanism of this strategy. Based on this principle, the better combining capacity of RRGGDD over RRDD can be attributed to the reduction of steric hindrance in peptides folding. Accordingly, **Scheme 1** shows the proposed Ag^{+} detection mechanism of the colorimetric assay.



Scheme 1. Schematic illustrations of colorimetric sensing mechanism based on Ag^{+}-induced aggregation of peptide-AuNPs.

3.2. Parameters optimization

We then estimated several parameters regarding the pH, the concentration of PB solution, the diameter of AuNPs and the reaction time of the mixture. The diameter of AuNPs employed in the experiment and concentration of PB were optimized and shown in **Fig. S4**. AuNPs with a diameter of ~30 nm were selected while 15 minutes for the reaction time were chosen.

The effect of pH on Ag^{+}-induced aggregation of peptide-AuNPs was investigated as well, which had important effects on the system. As shown in **Fig. S4c**, the ratio of absorption ($A_{700\ nm}/A_{550\ nm}$) in the presence (1 μM) and in the absence of Ag^{+} was

maximized at pH 7.4. In acidic media, the value of pH was lower than the isoelectric point of peptide I (pI 6.34), so the peptides were positively charged, resulting in the aggregation of negatively changed AuNPs. On the contrary, the peptides were negatively charged in alkaline media, potentially protecting AuNPs from aggregation. Therefore, pH of 7.4 was selected.

PB concentration plays an important role in the ion strength of the solution. The ratio of $A_{700\ nm}/A_{550\ nm}$ in the presence (1 μM) and absence of Ag^+ was shown in **Fig. S4d**. It was evident that below the concentration of 10 mM, the ratio of $A_{700\ nm}/A_{550\ nm}$ in the presence and absence of Ag^+ was small, since the peptide could stabilize AuNPs at low ionic strength. And the ratio increased as the concentration of Ag^+ increasing. Once the concentration of PB was more than 15 mM, the ratio of $A_{700\ nm}/A_{550\ nm}$ in the presence and absence of Ag^+ decreased due to the unstable peptide-AuNPs at high ionic strength. Here, the concentration of 10 mM or 15 mM was selected throughout the experiments.

3.3. Colorimetric assay for rapid Ag^+ sensing

Peptide-AuNPs were characterized by UV-vis spectroscopy and transmission electron microscopy (TEM). As shown in **Fig. 1**, a characteristic SPR peak of peptide-AuNPs was observed in the spectrum at approximate 550 nm (**Fig. 1A**) and the corresponding TEM observation was shown in **Fig. 1B(a)** and the peptide-AuNPs were dispersed. Upon addition of Ag^+ (800 nM) to peptide-AuNPs, the color of the solution turned purple due to the Ag^+-induced aggregation, further supported by TEM

image (**Fig. 1B(b)**).

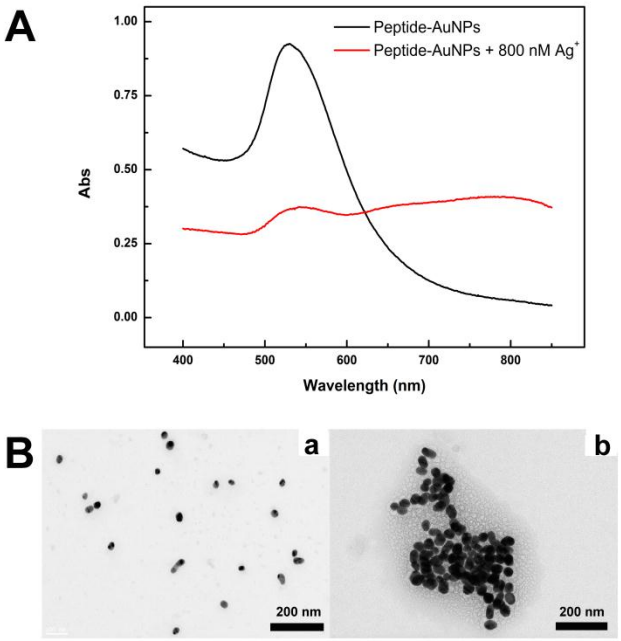


Fig. 1. (A) UV-vis spectra and (B) TEM images of the peptide-AuNPs in the (a) absence and (b) presence of 800 nM Ag^+ solution.

To determine the detection limit of the colorimetric assay, we prepared different concentrations of Ag^+ solutions from 10 to 1500 nM. As the Ag^+ concentrations increasing, the color of the AuNPs gradually changed from red to purple and finally to gray (**Fig. 2A**), indicating that the aggregation of peptide-AuNPs depended on Ag^+ concentrations. We used the ratio of absorption ($A_{700\ nm}/A_{550\ nm}$) to express the degree of the aggregation as mentioned above. **Fig. 2C** showed the concentration was dependent on the colorimetric response ($A_{700\ nm}/A_{550\ nm}$ values) of the assay. The $A_{700\ nm}/A_{550\ nm}$ values apparently increased with the Ag^+ concentrations ranging from 10 to 1000 nM. While Ag^+ concentrations increased to 1500 nM, the colorimetric response slightly increased. Definitely, the inset of **Fig. 2C** showed that the response of the

assay was extremely linear, with a linear regression correlation coefficient of 0.994 at the Ag^+ concentrations range of 10 to 1000 nM. The detection limit of Ag^+ with our system is proposed to be 7.4 nM, which is significantly below the allowed Ag^+ concentration limit (460 nM) defined by the United States Environmental Protection Agency (USEPA) in drinkable water (**Lin et al., 2011**). Our novel assay is one of the most sensitive and rapid colorimetric Ag^+ sensors compared with other colorimetric approaches (**Table S1**).

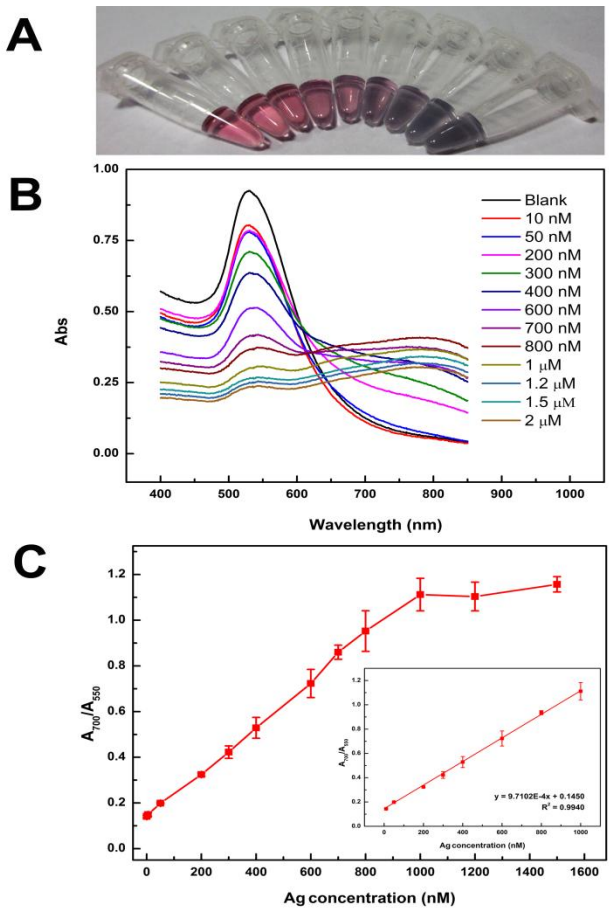


Fig. 2. Colorimetric detection of Ag^+. (A) Photo images of peptide-AuNPs after adding different concentrations of 0, 50, 200, 400, 700, 800, 1000, 1500, 2000 nM Ag^+ (from left to right). (B) Absorption spectra and (C) response ($A_{700\ nm}/A_{550\ nm}$ values) of the colorimetric assay against 10 mM PB (pH 7.4) containing

peptide-AuNPs and 0.1 mM EDTA upon the addition of increasing concentrations of Ag^+. Inset shows the response linearity of the assay at the Ag^+ concentrations range of 10 to 1000 nM.

3.4. Selectivity for determination of Ag^+

To identify the selectivity of the colorimetric assay against Ag^+, 14 cations (Cu^{2+}, Ni^{2+}, Pb^{2+}, Co^{2+}, Zn^{2+}, Ca^{2+}, Mn^{2+}, Mg^{2+}, K^+, Fe^{3+}, Cr^{3+}, Ag^+, Na^+, Li^+) and 7 anions (SO_4^{2-}, AcO^-, NO_2^-, F^-, CO_3^{2-}, Cl^-, NO_3^-) were tested. **Fig. 3** showed the response of the colorimetric assay against Ag^+ (1 μM) and other ions (10 μM) in the absence and the presence of EDTA. As expected, Ag^+ led to an evident increase in the $A_{700\ nm}/A_{550\ nm}$ values of the assay. Without EDTA, none of ions could cause the colorimetric response except for Hg^{2+}. Yet there was a slight response resulting from Hg^{2+}, which was considerably lower than the response from Ag^+ although the concentration of Hg^{2+} ions was 10 fold higher than the concentration of Ag^+. The reason for the slight colorimetric response of Hg^{2+} ions might be owing to their coordination with free -COOH groups of the aspartic acid. However, the interaction between -COOH groups and Hg^{2+} ions could be expected to be much weaker than the interaction of 4-coordinated complexes among -COOH groups, $-NH_2$ groups and Ag^+. Hence, a slight response resulted from Hg^{2+} occurred even at a high ion concentration. To further optimize the selectivity, EDTA was added into the peptide-AuNPs, which could mask the Hg^{2+} for Ag^+ detection. The results indicated the prominent selectivity of the colorimetric assay for Ag^+ ions (**Fig. 4**). Besides that, the influence of several

common anions, such as F^-, AcO^-, Cl^-, SO_4^{2-}, NO_3^-, NO_2^- and CO_3^{2-} were researched on Ag^+ binding to peptide-AuNPs. As shown in **Fig. S5**, the colorimetric response caused by the mixture of Ag^+ and another anion was similar to that caused by solely Ag^+ ions, which declared that tested anions had no interference on the determination of Ag^+.

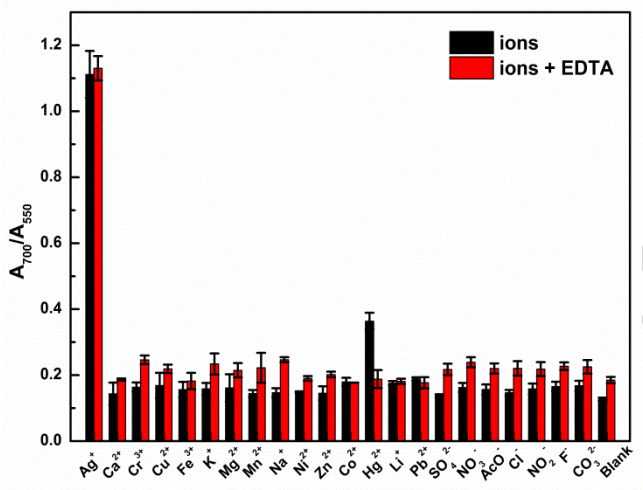


Fig. 3. Response ($A_{700\ nm}/A_{550\ nm}$ values) of peptide-AuNPs treated with several common ions (10 μM) and Ag^+ (1 μM) in the presence (0.1 mM) and absence of EDTA. The error bars represent standard deviations based on three independent measurements.

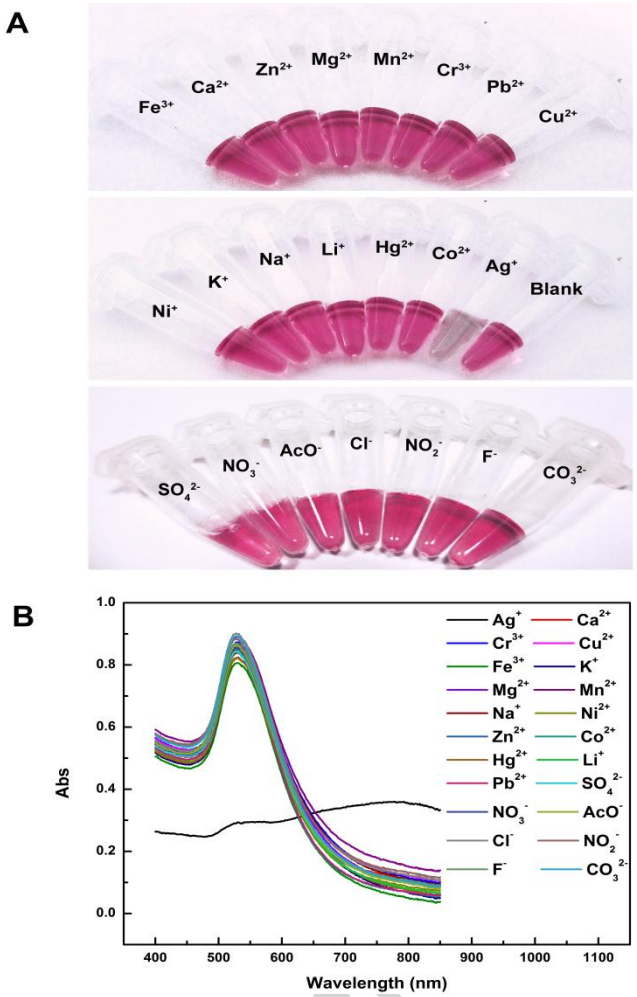


Fig. 4. Selectivity of the sensor. (A) Photo images and (B) the corresponding UV-vis absorption spectra of the peptide-AuNPs against several common ions (10 μM) and Ag^+ (1 μM) in the presence (0.1 mM) of EDTA.

3.5. Practical application

In certain environmental samples, such as lake water, the concentrations of some metal ions or some unknown pollutants are obviously higher than that of Ag^+, so potential practical assay is obligatory, and it is a crucial issue to the application of most common sensors. To confirm the practical application of the proposed approach, we prepared water samples from Donghu Lake and filtered through a 0.45 μm

membrane and then collected a series of samples by spiking them with different concentrations of Ag^+ (400, 600, 800 nM). The recovery results of Ag^+ in lake water samples were shown in **Table 1**, indicating great applicability of this sensor. Then, we successfully applied the approach to determinate the recovery of Ag^+ in tap water (**Table S2**) and drinking water (**Table S3**). Based on the experiment results, we believe that peptide-AuNPs can be employed as probes for Ag^+, which holds great potential in practical applications.

Sample (lake water)	**Ag^+ (added) nM**	**Ag^+ (detected) nM**	**Recovery (%)**	**RSD (%)**
1	400	428.05	107.01	9.60
2	600	600.41	100.07	6.61
3	800	839.68	104.96	2.50

Table 1. Results of the Ag^+ recovery experiments performed in lake water.

4. Conclusions

In brief, we proposed a new, simple, rapid, and extremely sensitive colorimetric assay for Ag^+ ion detection, which employed Ag^+ as an aggregation promoter for

peptide-AuNPs. The assay used the peptides to protect AuNPs from the aggregation. Upon the addition of Ag^+, peptide-AuNPs could undergo aggregating due to the interaction between the peptides and Ag^+. The detection limit of the colorimetric assay was approximately 7.4 nM, which is below the limit (460 nM) defined by the USEPA in drinking water. The assay response was highly linear in a wide concentrations range (10 to 1000 nM). Also, selectivity of the sensor was demonstrated in the presence of several common cations and anions at high concentrations (10-fold toward Ag^+). And all analyses could be accomplished within a few minutes. To our knowledge, few reports on determination of Ag^+ by the interaction between peptides and Ag^+ have been published so far. In our assay, we explored the principle of peptides and Ag^+, then applied it in the design of a novel, sensitive sensor for Ag^+ detection. Taken other superiorities of the system into consideration, such as simplicity, rapidness of the detecting process, superior sensitivity, and selectivity, great stability of peptide-AuNPs, our method is advantageous over other colorimetric approaches (**Table S1**) and prospective for on-site rapid monitoring of Ag^+.

Notes

Additional experimental results of comparison of colorimetric methods, possible explanations of the principle, peptides modification, verification process of this mechanism, optimization of relevant parameters and practical applications are presented in the Supporting Information.

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Appendix A. Supporting information

Supplementary data associated with this article can be found at the appendices.

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Hightlights

- Ag^{+}-triggered aggregation of peptide-AuNPs is newly proposed.
- The principle depends on the behavior of Ag^{+}-induced folding structure of peptides.
- Ultrasensitive detection of silver ions with potential as practical application in complex water samples.
- We present a simple, rapid, original method with excellent selectivity.