ACS APPLIED MATERIALS & INTERFACES

LETTER

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A Cu@Au Nanoparticle-Based Colorimetric Competition Assay for the Detection of Sulfide Anion and Cysteine

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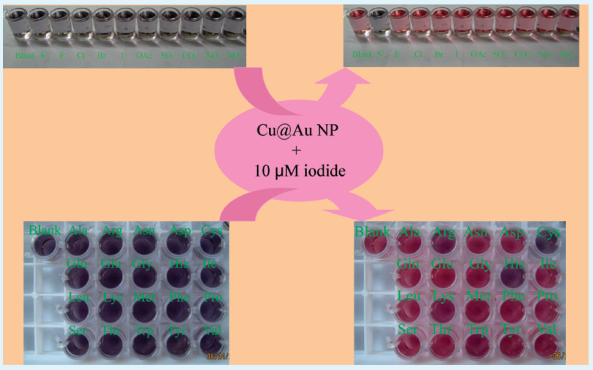
Supporting Information

ABSTRACT: As an extension of our previous work, which described the unique ability of the core/shell Cu@Au nanoparticle (NP) to selectively recognize iodide,[1] herein, we wish to report the development of an alternatively sensitive and selective colorimetric detection for sulfide anion and cysteine based upon the Cu@Au NP by a competition avenue. In the absence of sulfide anion or cysteine, iodide can induce an appreciable color change of the Cu@Au NP solution from purple to red by transforming the clusters of NP to single, nearly spherical, and larger ones. However, the transformation is severely interfered by the presence of sulfide or cysteine because of a higher binding strength of the S−Au bond than the I−Au one. As a result, the clear purple-to-red color change induced by iodide is affected as a correlation with the concentration of sulfide or cysteine. By taking advantage of this fact, we can detect a concentration of 3 μM for sulfide and 0.4 μM for cysteine with the naked eye or 0.3 μM (10 ppb) for sulfide and 50 nM (6 ppb) for cysteine aided by a UV/vis spectrometer. Given the detrimental effect of hydrogen sulfide and the biological importance of cysteine, the assay may become useful in the environment monitoring, water quality inspection and biomedical diagnosis as well.



KEYWORDS: anions, Cu@Au nanoparticle, colorimetry, competition assay, sulfide, cysteine

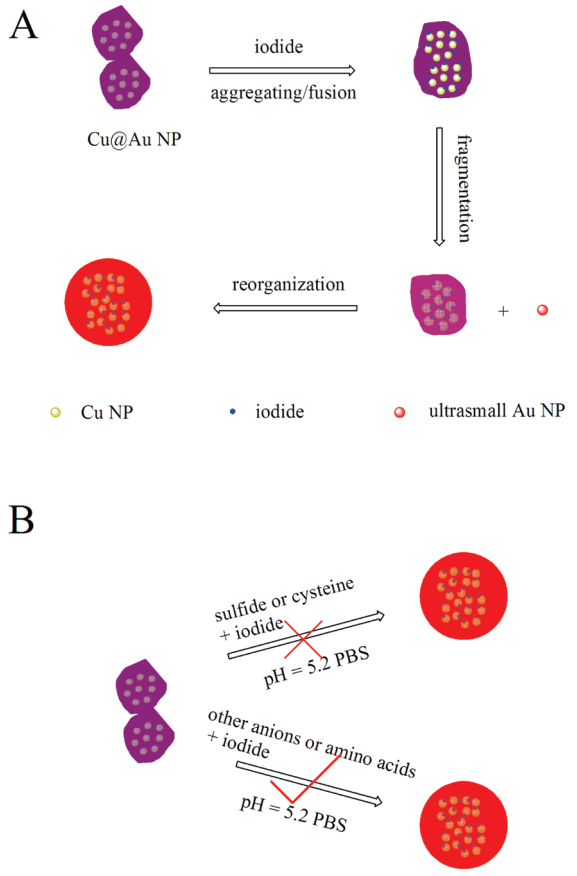
Anions are ubiquitous in the ecological circle, from Mother Nature to ordinary human lives. For the past decade, considerable progress has been made in the area of anions sensing and recognition since the detection of anions can not only provide a means for environment inspection and water quality examination but also contribute to the biomedical diagnosis of some diseases and offer an alternative perspective to explore the biological functions that the anions play in the human bodies.[2] Many strategies have been proposed for the detection of anions, whereas the most classic and applied approach is to employ supramolecular chemistry to design chromogenic or fluorogenic anion receptors which can guarantee a good or sometimes remarkably excellent selectivity.[3−5] However, the development of such receptors usually requires a whole set of sophisticated organic reactions and purification, and more unfavorably, their applications often suffer from rather high limit of detection. Recently, colorimetry by virtue of gold nanoparticles (Au NPs) emerges as a promising methodology for the anions sensing based on the high extinction coefficient and the distance-dependent optical property. Typical examples are the chemically functionalized spherical Au NP and Au nanorod for the selective recognition of nitrite anion.[6,7] Bearing the concept of colorimetry by nanoparticles in mind, very recently, we have devised a method for the highly specific recognition of iodide based on the core/shell Cu@Au nanoparticle.[1] No anions other than iodide can induce a geometric transformation of the Cu@Au NP, and the visible color change of solution can be seen from purple to red within 20 min (Scheme 1A).

Notorious for the repulsive odor as a noxious gas of its gaseous form (H_2S), sulfide is widely present in both natural water through bacterial immobilization of sulfur-containing minerals and wastewater mainly caused by a number of manufacturing or industrial processes.[8] Recently, it was discovered to be an endogenous signaling molecule responsible for the cardioprotective role.[9] Although the human nose can detect a minimum level of 0.02 ppm for H_2S in atmosphere, there is usually a threshold of ~5 ppm above which olfactory sense of the gas is severely impaired through prolonged exposure.[8] Many gas sensors have been devised to monitor volatile H_2S,[10−12] and meanwhile, several techniques are available for the detection of sulfide anion, such as spectrophotometry,[13] fluorometry,[14] and electrochemistry.[15] Although an impressive number of methods for testing sulfide or hydrogen sulfide exist, a simple, rapid, and accurate test for dangerous levels of sulfide is urgently needed,

Received: June 14, 2011
Accepted: July 25, 2011
Published: July 25, 2011

dx.doi.org/10.1021/am2007678 | ACS Appl. Mater. Interfaces 2011, 3, 2928–2931

Scheme 1. (A) Representation of the Iodide-Induced Geometric Transformation of the Cu@Au NP; (B) Representation of Interference of the Iodide-Induced Geometric Transformation of the Cu@Au NP by Sulfide or Cysteine, Which Constitutes the Basic Principle for the Sensing of Sulfide or Cysteine



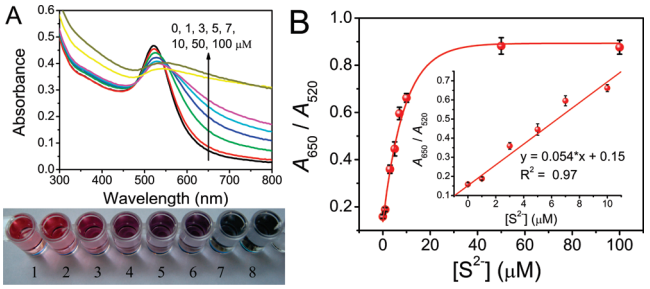


Figure 1. (A) UV/vis spectra (above) of the buffered Cu@Au NP solution containing 10 μM iodide and different concentrations of sulfide from 0 to 100 μM, pH 5.2; colorimetric visualization (bottom) of sulfide corresponding to the optical spectra, with concentrations of the samples marked with 1−8 being 0, 1, 3, 5, 7, 10, 50, and 100 μM, respectively. (B) Variation plot of A_{650}/A_{520} against the concentration of sulfide. The inset is an enlargement of the plot among the concentration range from 0 to 10 μM, with the error bars representing the standard deviations of three parallel measurements.

because the present methods are largely limited in rural and remote workplaces and areas where evaluation is most essential.

Extended by our previous work[1] here, we demonstrate the colorimetric detection of sulfide anion and one sulfur-containing amino acid, i.e., cysteine, on the basis of the Cu@Au NP (Scheme 1B). The assay is developed on a competitive adsorption mechanism. It was found that the colorimetric recognition of iodide was greatly interfered by the presence of sulfide, suggesting that sulfide is adsorbed to the surface of Cu@Au NP more strongly than iodide. In this manner, sulfide can be alternatively detected by the same nanoparticle originally developed to specifically recognize iodide. Moreover, we also managed to use it for the cysteine sensing among a range of amino acids with a satisfactory result.

The Cu@Au NP was prepared by following the previous procedure (Experimental, Supporting Information). To test the idea of the competition assay for sulfide, in a typical experiment, the buffered Cu@Au NP solution (150 μL NP + 150 μL PBS buffer, pH 5.2) were initially added with several concentrations of sulfide, homogeneously mixed and incubated for 5 min, followed by the addition of iodide (10 μM) for another 10 min before the optical spectra were collected. It is appreciated that sulfide exists in three species (H_2S, HS^-, and S^{2-}) in aqueous solution defined by its pK_a,[16] and under the present pH status, it exists as H_2S dominantly. However, herein, the term sulfide refers to totality of the three species. A pH of 5.2 was selected, since the most preferable recognition performance on iodide occurred at this pH status among the range from 5.2 to 9.0, as explained in the previous study.[1] Figure 1A shows the optical spectra and color of the Cu@Au NP solutions containing sulfide of different concentrations and iodide after the 15 min of interaction. The clear spectral change is attributed to the differed degree of particle transformation, indicating the stronger binding affinity of sulfide than iodide toward the surface of the Cu@Au NP. Besides, the color of the solution varied appreciably from red (0, 1 μM) through purple reddish (3, 5 μM) and purple (7, 10 μM) to blue (50, 100 μM). The coaddition of sulfide and iodide could also lead to the analogous colorimetric response, whereas such spectacular change was not observed when the addition of iodide was preceded before that of sulfide under the same otherwise conditions. The sequence of addition for iodide and sulfide is of such crucial importance for the sulfide assay in that the Cu@Au NP after recognizing iodide becomes dynamically stable, thus no interference effect will occur after the successive addition of sulfide. By correlating the absorbance ratio at 650−520 nm (A_{650}/A_{520}), a function curve of first-order exponential decay is obtained, as shown in Figure 1B. A linear relationship can be inferred (inset in Figure 1B), manifesting the dynamic range from 0 to 10 μM and a limit of detection (LOD) being 0.3 μM (3σ). Such performance characteristics compare favorably to most of existing spectroscopic or electrochemical methods,[8,13−15] and the facile readout, necessitating no toxic organic reagents as well as the convenient preparation procedure are also featuring the advantages of the sensor. The storing stability of the sensor or the Cu@Au NP was found to decrease with the elevation of temperature. Under the present situation throughout the experiment (20 ± 2 °C), the sensor was stable for at least 2 weeks before sedimentation of particles was observed.

Prior to potential application of the promising assay for the sulfide, selectivity must be evaluated, by challenging it with other environmentally relevant anions. All of the tested anions (100 μM) can not elicit a response similar to sulfide (10 μM) except thiosulfate, which can cause a false positive result (see Figure S1 in the Supporting Information). Meanwhile, the assay will not be interfered by the coexistence of other anions with the

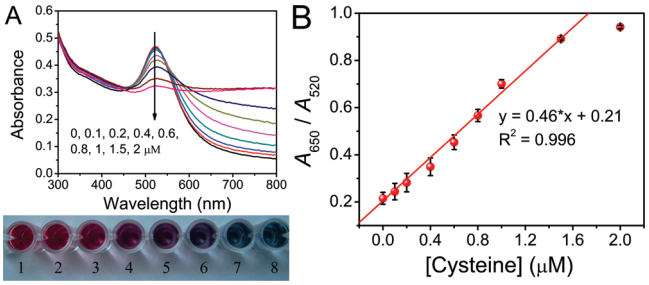


Figure 2. (A) UV/vis spectra (above) of the buffered Cu@Au NP solution containing 10 μM iodide and different concentrations of cysteine from 0 to 2 μM, pH 5.2; colorimetric visualization (bottom) of sulfide corresponding to the optical spectra, with concentrations of the samples marked with 1−8 being 0, 0.2, 0.4, 0.6, 0.8, 1, 1.5, and 2 μM, respectively. (B) Variation plot of A_{650}/A_{520} against the concentration of cysteine, with the error bars representing the standard deviations of three parallel measurements.

same exception of thiosulfate. It should also be mentioned that the test suffers little interference from the metallic cations typically present in water, such as Ca^{2+} and Mg^{2+}, at concentrations of 100 μM. Above the concentration, interference will become more obvious, yet some strong chelating compound, such as EDTA, can help to minimize the possible interference to the least. Taken together, the Cu@Au NP can serve as a promising platform for the quantitative determination of sulfide anion in water.

Importantly, we further extended the sulfide-selective competition ability of the Cu@Au NP system to the detection of cysteine among a range of amino acids with a satisfactory result. Amino acids are one kind of building blocks in living systems, joining together to yield proteins, enzymes, structural elements, and many other molecules of biological activity. As a sulfur-containing amino acid, cysteine has been identified to be a biomarker for many medical syndromes[17] and a physiological regulator associated with some diseases.[18] Of such significance, great enthusiasm has been devoted to the determination of cysteine and plenty of strategies have thus been developed, such as colorimetry or fluorometry based on chromophores[19] or fluorophores,[20] electrochemical voltammetry,[21] and the use of modern, massive, and expensive analytical equipment.[22] Recently, colorimetry or fluorometry by virtue of the high affinity of the biothiol toward Hg^{2+} ion emerges as a new and sensitive methodology.[23−25] The possible drawback, as is apparent, is the utility of environmentally hazardous cation (Hg^{2+}).

From the above investigation, we surmise that if sulfide can suppress the iodide-induced geometric transformation of the Cu@Au NP, so can cysteine. To test whether the capability of competition-based sulfide assay can be applied to the cysteine sensing, we made some changes, replacing sulfide with cysteine into the Cu@Au NP solution, with the other conditions and procedures unvaried. Analogous optical responses as those of sulfide anion were expected, as shown in Figure 2A, supporting the concept of the potential extension of the colorimetric system to the cysteine assay. Notable color change of solution was also observed, from red (0, 0.2 μM) through purple reddish (0.4, 0.6 μM) and purple (0.8, 1 μM) to blue (1.5, 2 μM). Figure 2B shows the correlation between the derived A_{650}/A_{520} ratio and the concentration of cysteine. A linear relationship was obtained in the response range from 0 to 1.5 μM, with a LOD being 50 nM (3σ). Such performance characteristics are comparable to or even better than those of many present methods, whereas the superiority of the method lies in its simple procedures and facile readout. Moreover, the selectivity of the assay was tested by the other 19 natural amino acids (10 μM) against cysteine (1 μM) (see Figure S2 in the Supporting Information). It is clear that the colorimetric system exhibits satisfactory selectivity toward cysteine, with histidine and arginine as the first and second largest interference, and others insignificant interferences, including the sulfur-containing methionine. When we reduced the concentrations of histidine and arginine down to 1 μM, both interferences can be neglected. Two other small biothiols, i.e., homocysteine and glutathione, which also play crucial roles in maintaining biological systems,[26] are not considered in this Letter; however, we tend to think that they can be detected in the same competition avenue because they have the similar structures as cysteine.

In conclusion, as an extension of our previous work, this manuscript describes a new type of simple, selective, and highly sensitive colorimetric assay for detecting sulfide anion and cysteine using the Cu@Au NP that was originally prepared for the specific recognition of iodide. This assay is based on a competitive adsorption concept, by which sulfide anion or the biothiol molecule adsorbs more strongly than iodide on the surface of the Cu@Au NP. As a result, differed degree of particle transformation induced by iodide will be correlative with the concentrations of sulfide or cysteine, which can be conveniently reflected by the optical spectra with a UV/vis spectrometer or the color change of solution. Taking advantage of this competition assay format, we can easily detect 3 μM (0.1 ppm) of sulfide and 0.4 μM (48 ppb) of cysteine with the naked eye or 10 ppb of sulfide and 6 ppb of cysteine with a UV/vis spectrometer. Besides the high sensitivity and low detection limit, the sensor manifests good stability, reproducibility, and interference-invulnerable capability. Given the pressing demand for rapid monitoring of sulfide anion or hydrogen sulfide and the biological importance of cysteine, this assay may be applied in the environment inspection and biomedical research community.

■ ASSOCIATED CONTENT

Supporting Information. Experimental section and additional figures as noted in the text. This material is available free of charge via the Internet at http://pubs.acs.org.

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■ ACKNOWLEDGMENT

This work was supported by the National Natural Science Foundation of China (20890020) and the National Key Basic Research Development Project of China (2010CB933602; 2007CB714500).

■ REFERENCES

(1) Zhang, J.; Xu, X.; Yang, C.; Yang, F.; Yang, X. *Anal. Chem.* **2011**, *83*, 3911–3917.

(2) Xu, Y.; Szép, S.; Lu, Z. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 20515–20519.

(3) Wade, C. R.; Broomsgrove, A. E. J.; Aldridge, S.; Gabbaï, F. P. *Chem. Rev.* **2010**, *110*, 3958–3984.

(4) Xu, Z.; Chen, X.; Kim, H. N.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 127–137.
(5) Kim, S. K.; Lee, D. H.; Hong, J.; Yoon, J. *Acc. Chem. Res.* **2009**, *42*, 23–31.
(6) Daniel, W. L.; Han, M.; Lee, J.; Mirkin, C. A. *J. Am. Chem. Soc.* **2009**, *131*, 6362–6363.
(7) Xiao, N.; Yu, C. *Anal. Chem.* **2010**, *82*, 3659–3663.
(8) Lawrence, N. S.; Davis, J.; Compton, R. G. *Talanta* **2000**, *52*, 771–784.
(9) Benavides, G. A.; Squadrito, G. L.; Mills, R. W.; Patel, H. D.; Isbell, T. S.; Patel, R. P.; Darley-Usmar, V. M.; Doeller, J. E.; Kraus, D. W. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 17977–17982.
(10) Mubeen, S.; Zhang, T.; Chartuprayoon, N.; Rheem, Y.; Mulchandani, A.; Myung, N. V.; Deshusses, M. A. *Anal. Chem.* **2010**, *82*, 250–257.
(11) Sen, A.; Albarella, J. D.; Carey, J. R.; Kim, P.; McNamara, W. B., III *Sens. Actuators B* **2008**, *134*, 234–237.
(12) Wallace, K. J.; Cordero, S. R.; Tan, C. P.; Lynch, V. M.; Anslyn, E. V. *Sens. Actuators B* **2007**, *120*, 362–367.
(13) Kubáň, V.; Dasgupta, P. K.; Marx, J. N. *Anal. Chem.* **1992**, *64*, 36–43.
(14) Yang, X.; Wang, L.; Xu, H.; Zhao, M. *Anal. Chim. Acta* **2009**, *631*, 91–95.
(15) Lawrence, N. S. *Electroanalysis* **2006**, *18*, 1658–1663.
(16) Harris, D. C. *Quantitative Chemical Analysis*, 7th ed.; W. H. Freeman & Co.: New York, 2006, AP14.
(17) Goodman, M. T.; McDuffie, K.; Hernandez, B.; Wilkens, L. R.; Selhub, J. *Cancer* **2000**, *89*, 376–382.
(18) Gazit, V.; Ben-Abraham, R.; Coleman, R.; Weizman, A.; Katz, Y. *Amino Acids* **2004**, *26*, 163–168.
(19) Huo, F.; Sun, Y.; Su, J.; Chao, J.; Zhi, H.; Yin, C. *Org. Lett.* **2009**, *11*, 4918–4921.
(20) Chen, X.; Ko, S.-K.; Kim, M. J.; Shin, I.; Yoon, J. *Chem. Commun.* **2004**, *46*, 2571–2573.
(21) Zhou, M.; Ding, J.; Guo, L.; Shang, Q. *Anal. Chem.* **2007**, *79*, 5328–5335.
(22) Gegg, M. E.; Clark, J. B.; Heales, S. J. *Anal. Biochem.* **2002**, *304*, 26–32.
(23) Lee, J. S.; Ulmann, P. A.; Han, M. S.; Mirkin, C. A. *Nano Lett.* **2008**, *8*, 529–533.
(24) Pu, F.; Huang, Z.; Ren, J.; Qu, X. *Anal. Chem.* **2010**, *82*, 8211–8216.
(25) Xu, H.; Hepel, M. *Anal. Chem.* **2011**, *83*, 813–819.
(26) Chem, X.; Zhou, Y.; Peng, X.; Yoon, J. *Chem. Soc. Rev.* **2010**, *39*, 2020–2135.