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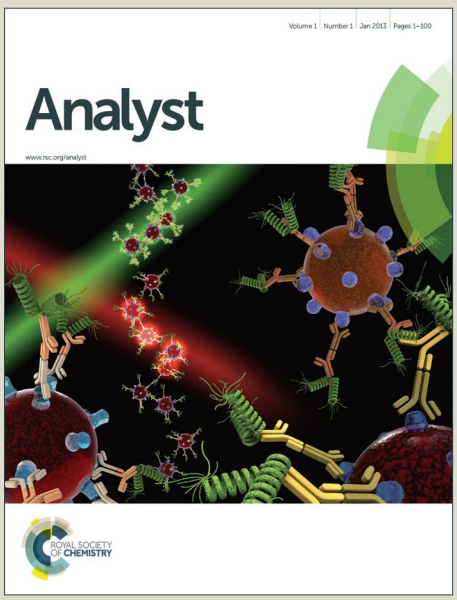

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A highly sensitive colorimetric metalloimmunoassay based on copper-mediated etching of gold nanorods†

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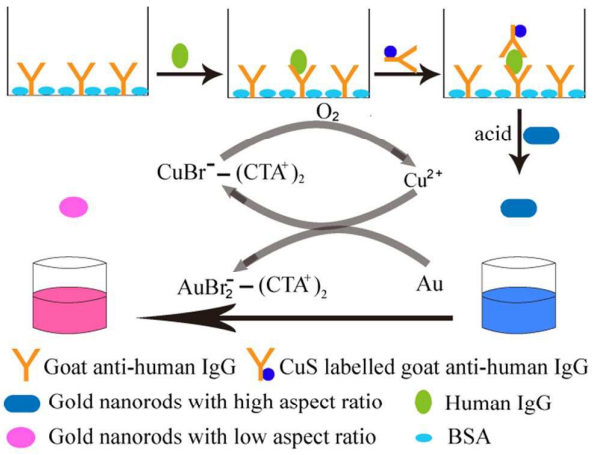
A highly sensitive colorimetric metalloimmunoassay with a detection limit of 0.15 ng/ml for human IgG based on copper-mediated etching of gold nanorods was proposed. The assay is more sensitive than traditional ELISA, electrochemical metalloimmunoassay and HRP mimic nanomaterial tags-based immunoassay.

Ultrasensitive sensing of biomolecules and metal ions, such as biomarker, dopamine, cysteine, Cu^{2+}, Hg^{2+}, Cd^{2+}, Pb^{2+}, etc. plays very important role in diverse areas, including clinical diagnosis, food control and environmental monitoring.[1-7] For this purpose, many analytical technologies were developed, including nonmaterial-based colorimetry and fluorometry, paper-based fluorometry, electrochemistry, atomic spectrometry, immunoassay, etc. Among these, immunoassay was proven to be an effective and ultimately successful method for the quantification of target analytes.[8-11] Due to its long history, much more mature operation process and plenty of encouraging experimental results, enzyme-linked immunosorbent assay (ELISA) remains the most popular clinical detection approach.[12-15] However, ELISA still suffers from the inherent shortcomings of antigen/antibody-protein conjugates, such as easy denaturation and digestion by proteases, the cumbersome production process and time-critical storage life.[16-18] To overcome these shortcomings, much efforts have focused on the discovering better labels to conjugate antigen or antibody.[16, 19-22] Due to its unique physical and biochemical properties, metal nanomaterials have proved to be effective tags in indicating the reaction between antigen and antibody. Normally, the metal nanomaterial tags are quantifed by electrochemistry, chemiluminescence, etc.[23-27] Although these quantitative measurements provide some advantages, such as low sample volume, excellent sensitivity and relatively inexpensive instrumentation, the promotion and application of these technologies are constrained by their complicated procedures and rigorous experimental details. Nowadays, spectrophotometric immunoassay remains the dominant position as before for most hospitals and disease surveillance organizations, even in the remote poor areas or countries, possess microplate readers and spectrophotometers rather than electrochemical or chemiluminescent instruments. So it is necessary to develop effective methods that can sensitively read nanomaterials tags using microplate readers or spectrophotometers. For this purpose, many HRP mimic nanomaterial tags, such as graphene, Au@Pt, Pt@SiO_2 and platinum nanoparticles have been applied to immunoassay.[16, 28-36] These methods have also obtained high sensitivity that are comparable with ELISA due to the high catalytic activity of the HRP mimics.

Methods based on catalytic etching of gold nanoparticles



Scheme 1. The schematic illustration of procedure of the colorimetric metalloimmunoassay

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have been proved to be simple and sensitive for colorimetric detection of many target analytes, including Pb^{2+}, Cu^{2+}, Co^{2+}, MoO_4^{2-}, etc.[37-40] In this work, we combine the conventional metalloimmunoassay with copper-mediated etching of gold nanoparticles proposed in our previous work to develop an effective immunoassay using a microplate reader or a spectrophotometer as readout. The experiment results show the immunoassay is more sensitive than traditional ELISA and HRP mimic nanomaterial tags-based immunoassay.[31, 34, 41-42]

Scheme 1 outlines the procedure of the colorimetric metalloimmunoassay based on copper-mediated etching of gold nanorods using human IgG as model antigen. Goat anti-human IgG was first adsorbed onto polystyrene microplate physically. After a conventional sandwich immunoreaction, different amounts of CuS nanocrystals labelled goat anti-human IgG proportional to the target analyte (human IgG) was captured onto the microplate (TEM image shows that the spherical CuS nanocrystals is about 7 nm and the X-ray diffraction pattern indicates the synthesized CuS nanocrystals is in high quality. Fig. S1 and S2 in ESI†). The cupric ion (Cu^{2+}) was then released by the addition of hydrobromic acid and was determined based on the catalytic etching of gold nanorods.

Fig. 1A shows the changes of the the LSPR absorption (A), and color (inset) of gold nanorods induced by different concentrations of Cu^{2+} which comes from the captured CuS nanocrystals in the sandwich immunoreaction. The longitudinal LSPR absorption peaks located at 668, 596 and 555 nm respectively after the incubation of gold nanorods with different concentrations of human IgG-mediated Cu^{2+}. The solution of gold nanorods also changed from blue to purple

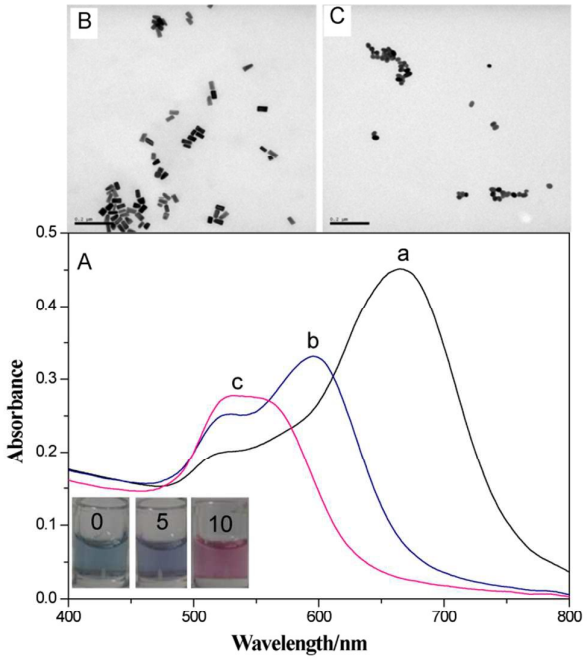

Fig. 1. LSPR absorption spectra (A) and the colors (inset) respond to 0 (a), 5 (b) and 10 ng/ml (c) human IgG. B and C are the TEM images of gold nanorods responding to 0 (B) and 10 ng/ml (C) human IgG.

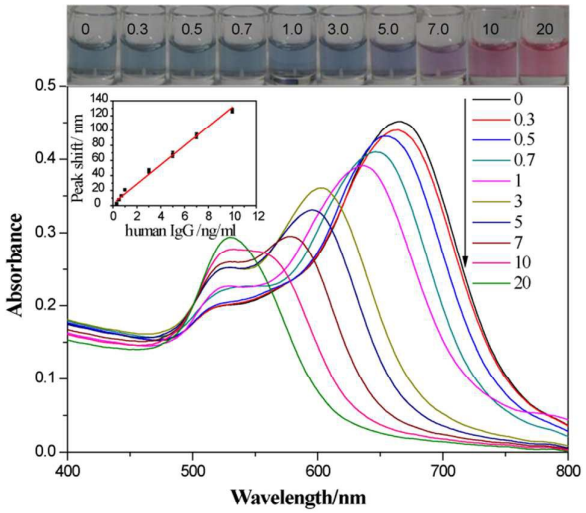

Fig. 2. LSPR absorption spectra and the colors of gold nanorods respond to different concentration of human IgG (ng/ml). The inset is the longitudinal LSPR peak-shift vs the concentration of human IgG (ng/ml).

and then to red with the increasing of the concentration of human IgG. The peak-shift and color change are both owning to the anisotropic corrosion of gold nanorods along the longitudinal direction since the less surface passivation and/or the higher reaction activities at the tips (Fig. 1B and C).[43] Obviously, the peak-shift and color change can be applied to indicate the target analyte concentration.

It should be noted here that our previous work had not investigated the oxidation product of gold nanorods.[40] Here we used mass spectrometry to identify the oxidation product. As shown in Fig. S3 in ESI†), the appearance of a peak at m/z 925 indicated the formation of $AuBr_2^-$–$(CTA^+)_2$ where CTA^+ was the abbreviation of cetyltrimethylammonium. The formation of $AuBr_2^-$–$(CTA^+)_2$, an ionassociation compound, can be explained by the soft and hard acid–base theory that cation with large volume tends to associate with anion with large volume.[43] According to our previous work and the mass spectrometry experimental results, we conclude the colorimetric sensing of the captured CuS is based on the following chemical equations.

$$CuS + 2H^+ \rightarrow Cu^{2+} + H_2S$$

$$Cu^{2+} + Au + 4CTA^+ + 4Br^- \rightarrow CuBr_2^-–(CTA^+)_2 + AuBr_2^-–(CTA^+)_2$$

$$4CuBr_2^-–(CTA^+)_2 + O_2 + 4H^+ \rightarrow Cu^{2+} + 8Br^- + 8CTA^+ + 2H_2O$$

Fig. 2 shows the LSPR absorption spectra and the colors of gold nanorods responding to different concentraton of human IgG. The longitudinal LSPR peak shifted to short wavelength gradually with the increase in human IgG concentration in the range of 0.3 – 20 ng/ml, companied by a color change from blue to purple and then to red. The LSPR peak-shift could easily be monitored by a simple microplate reader or a spectrophotometer. The detection limit was calculated to be 0.15 ng/ml according to the S/N = 3 rule which is lower than the traditional ELISA, HRP mimic nanomaterial tags-based

Table 1. Comparison of immunoassay methods developed for IgG

label	immunoassay format	analytical technique	detection limit, ng/ml	ref
HRP	ELISA	spectrophotometry	1.0	34
HPR	immunomagnetic beads/sandwich	spectrophotometry	2.4	42
Ap	ELISA	spectrophotometry	1.0	41
Ap	ELISA	luminescence	0.03	41
Au	microwells/sandwich	strippingvoltammetry	0.5	23
CdS	immunomagnetic beads/sandwich	strippingvoltammetry	10	24
Au	capillary electrophoresis/homogeneous	chemiluminescence	1.14	25
Au/HRP	immunomagnetic beads/ sandwich	Strippingvoltammetry	0.26	42
Au/HRP	immunomagnetic beads/ sandwich	spectrophotometry	0.05	42
Eu-BCPDA	microwells/sandwich	time-resolved fluorescence	0.1	41
Pt	microwells/sandwich	spectrophotometry	2.5	34
Si@Pt	microwells/sandwich	spectrophotometry	10	31
CuS	microwells/sandwich	spectrophotometry	0.15	This work

immunoassay and electrochemical metalloimmunoassay and is comparable with fluorescent immunoassay (Table 1). The digital photo (Fig. 2B) shows low as 5.0 ng/ml human IgG can be detected by naked eyes. The eye-readable color change also makes the metalloimmunoassay more convenient in clinical diagnosis, avoiding the use of complex equipments.

High tolerance to interferents and good specificity of immunoassay can ensure the selective identification of target analyte from complex matrix. Fig. 3 shows the specificity and the tolerance of the proposed immunoassay. The almost no obvious peak shift for the respective determination of 1.0 mg/ml lactoglobulin, glucose, creatinine, glutamine, ascorbic acid, cysteine, catechin, papain, trypsin, peptone and glycine, and 1.0 μM Ca^{2+}, Mg^{2+}, Al^{3+}, Zn^{2+}, Fe^{3+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, Ag^{+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, NO_3^-, SO_4^{2-} and the mixture of these chemicals indicated the metalloimmunoassay displayed good specificity. The almost the same change in the LSPR of gold nanorods for the diterminination of 10 ng/ml IgG in the the presence and absence of the mixture of the above mentioned chemicals indicated the proposed metalloimmunoassay possessed high tolerance to those chemicals.

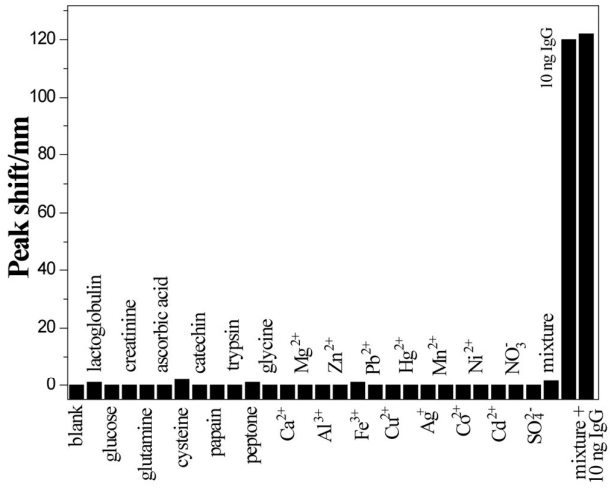

Fig. 3. LSPR peak shift of gold nanorods responds to 1.0 mg/ml lactoglobulin, glucose, creatinine, glutamine, ascorbic acid, cysteine, catechin, papain, trypsin, peptone and glycine, and 1.0 μM Ca^{2+}, Mg^{2+}, Al^{3+}, Zn^{2+}, Fe^{3+}, Pb^{2+}, Cu^{2+}, Hg^{2+}, Ag^{+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cd^{2+}, NO_3^-, SO_4^{2-}, the mixture of these chemicals , 10 ng/ml human IgG.

Commonly, immunoassay is performed in serum, which requires the abundance of proteins, salt and other small molecules, such as glucose, ascorbic acid, etc. do not interfer the detection. Since the normal concentration of human IgG in human serum reaches mg/mL levels, so the direct detection of human IgG in diluted human serum cannot evaluate the specificity of the proposed immunoassay. In consideration of the concentrations of proteins, salts and other small molecules in animal serum are comparable to human serum, here we choose the determination of spiked human IgG in fetal bovine serum (FBS) to investigate the specificity. The detection results are shown in Table 2. The recoveries of the spiked human IgG ranged from 104.2% to 106.7%. The satisfactory recoveries for determination low concentrations of human IgG indicate that such a method has good specificity and is applicable to the quantification of low abundant protein in complex biological sample, avoiding the interference from proteins, salts and other small molecules.

Table 2. Determination results in the spiked sample by the proposed metalloimmunoassay

Samples	Added, ng/ml	Found, ng/ml	Recovery
FBS	0.7	0.73 ± 0.02	104.3 ± 2.8
	3.0	3.2 ± 0.1	106.7 ± 3.3
	7.0	7.3 ± 0.4	104.2 ± 5.7

In conclusion, here we have developed a highly sensitive colorimetric metalloimmunoassay based on copper-mediated etching of gold nanorods. Due to cupric ion can accelerate the etching of gold nanorods by dissolved oxygen dramatically, the amount of captured CuS nanocrystals in sandwich immunoreaction process can be monitored by using a simple microplate reader, spectrophotometer and even by using naked eyes for the assay of target analyte with relative high concentrations, making the metalloimmunoassay easier and more convenient. The metalloimmunoassay is also more sensitive than traditional ELISA, electrochemical immunoassay and HRP mimic nanomaterial tags-based immunoassay and

comparable with fluorescent immunoassay. The good specificity comfirmed by the determination of human IgG in spiked samples indicates the metalloimmunoassay is applicable to the quantification of target analyte in complex samples. In the consideration of the pervasive application of immunoassay, the metalloimmunoassay promises to be an effective approach in clinical diagnosis and environment analysis for detection of biomarkers and contaminant, such as heavy metal ions, pesticides and pathogenic bacteria.

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Notes and references

1 Y. Zhang, H. Li, L.-Y. Niu, Q.-Z. Yang, Y.-F. Guan and L. Feng, *Analyst,* 2014, **139**, 3146.
2 S.-X. Li, X. Lin, F.-Y. Zheng, W. Liang, Y. Zhong and J. Cai, *Anal. Chem.,* 2014, **86**, 7079.
3 B. Jiang, F. Li, C. Yang, J. Xie, Y. Xiang and R. Yuan, *Anal. Chem.,* 2015, **87**, 3094.
4 L.-Y. Niu, M.-Y. Jia, P.-Z. Chen, Y.-Z. Chen, Y. Zhang, L.-Z. Wu, C.-F. Duan, C.-H. Tung, Y.-F. Guan, L. Feng and Q.-Z. Yang, *Rsc Adv.,* 2015, **5**, 13042.
5 H. Zhang, Y. Huang, S. Hu, Q. Huang, C. Wei, W. Zhang, L. Kang, Z. Huang and A. Hao, *J. Mater. Chem. C,* 2015, **3**, 2093.
6 Q. Huang, X. Lin, C. Lin, Y. Zhang, S. Hu and C. Wei, *Rsc Adv.,* 2015, **5**, 54102.
7 L. Feng, Y. Zhang, L. Wen, L. Chen, Z. Shen and Y. Guan, *Analyst,* 2011, **136**, 4197.
8 S. Blumenthal, *Perspect. Biol. Med.,* 2009, **52**, 343.
9 R. Ekins and D. Kelso, *Clin. Chem.,* 2011, **57**, 372.
10 J. P. Gaut, D. L. Crimmins, M. F. Ohlendorf, C. M. Lockwood, T. A. Griest, N. A. Brada, M. Hoshi, B. Sato, R. S. Hotchkiss, S. Jain and J. H. Ladenson, *Clin. Chem.,* 2014, **60**, 747.
11 X. Liu, Q. Dai, L. Austin, J. Coutts, G. Knowles, J. Zou, H. Chen and Q. Huo, *J. Am. Chem. Soc.,* 2008, **130**, 2780.
12 S. D. Gan and K. R. Patel, *J. Invest. Dermatol.,* 2013, **133**, e12.
13 B. Nilsson, *Curr. Opin. Immunol.,* 1989, **2**, 898.
14 T. Porstmann and S. T. Kiessig, *J. Immunol. Methods,* 1992, **150**, 5.
15 R. M. Lequin, *Clin. Chem.,* 2005, **51**, 2415.
16 J. Shen, Y. Li, H. Gu, F. Xia and X. Zuo, *Chem. Rev.,* 2014, **114**, 7631.
17 L. Z. Gao, J. M. Wu, S. Lyle, K. Zehr, L. L. Cao and D. Gao, *J. Phys. Chem. C,* 2008, **112**, 17357.
18 J. D. Conradie, M. Govender and L. Visser, *J. Immunol. Methods,* 1983, **59**, 289.
19 J. Tang and D. P. Tang, *Microchim. Acta,* 2015, **182**, 2077.
20 S. L. Zhu, Y. Q. Fu and J. Z. Hou, *J. Comput. Theor. Nanos.,* 2010, **7**, 1855.
21 N. L. Rosi and C. A. Mirkin, *Chem. Rev.,* 2005, **105**, 1547.
22 H. A. Ho, A. Najari and M. Leclerc, *Acc. Chem. Res.,* 2008, **41**, 168.
23 M. Dequaire, C. Degrand and B. Limoges, *Anal. Chem.,* 2000, **72**, 5521.
24 G. Liu, J. Wang, J. Kim, M. R. Jan and G. E. Collins, *Anal. Chem.,* 2004, **76**, 7126.
25 Y. M. Liu, L. Mei, L. J. Liu, L. F. Peng, Y. H. Chen and S. W. Ren, *Anal. Chem.,* 2011, **83**, 1137.
26 N. H. Tung, M. Chikae, Y. Ukita, P. H. Viet and Y. Takamura, *Anal. Chem.,* 2012, **84**, 1210.
27 Y. Wang, L. J. Tang and J. H. Jiang, *Anal. Chem.,* 2013, **85**, 9213.
28 W. He, Y. Liu, J. Yuan, J. J. Yin, X. Wu, X. Hu, K. Zhang, J. Liu, C. Chen, Y. Ji and Y. Guo, *Biomaterials,* 2011, **32**, 1139.
29 Z. X. Zhang, Z. J. Wang, X. L. Wang and X. R. Yang, *Sen. Actuat. B,* 2010, **147**, 428.
30 Z. Gao, M. Xu, M. Lu, G. Chen and D. Tang, *Biosens. Bioelectron.,* 2015, **70**, 194.
31 Z. Wang, X. Yang, J. Yang, Y. Jiang and N. He, *Anal. Chim. Acta,* 2015, **862**, 53.
32 L. Gao, J. Zhuang, L. Nie, J. Zhang, Y. Zhang, N. Gu, T. Wang, J. Feng, D. Yang, S. Perrett and X. Yan, *Nat. Nanotechnol.,* 2007, **2**, 577.
33 Y. C. Lee, M. I. Kim, M. A. Woo, H. G. Park and J. I. Han, *Biosens. Bioelectron.,* 2013, **42**, 373.
34 Z. Gao, M. Xu, L. Hou, G. Chen and D. Tang, *Anal. Chim. Acta,* 2013, **776**, 79.
35 L. Zhan, C. M. Li, W. B. Wu and C. Z. Huang, *Chem. Commun.,* 2014, **50**, 11526.
36 X. Que, D. Tang, B. Xia, M. Lu and D. Tang, *Anal. Chim. Acta,* 2014, **830**, 42.
37 Y. Y. Chen, H. T. Chang, Y. C. Shiang, Y. L. Hung, C. K. Chiang and C. C. Huang, *Anal. Chem.,* 2009, **81**, 9433.
38 Z. Zhang, Z. Chen and L. Chen, *Langmuir,* 2015, **31**, 9253.
39 Z. Zhang, Z. Chen, D. Pan and L. Chen, *Langmuir,* 2015, **31**, 643.
40 Z. Zhang, Z. Chen, C. Qu and L. Chen, *Langmuir,* 2014, **30**, 3625.
41 R. A. Evangelista, A. Pollak and E. F. G. Templeton, *Anal. Biochem.,* 1991, **197**, 213.
42 A. Ambrosi, M. T. Castaneda, A. J. Killard, M. R. Smyth, S. Alegret and A. Merkoci, *AnaL. Chem.,* 2007, **79**, 5232.
43 J. Rodriguez-Fernandez, J. Perez-Juste, P. Mulvaney and L. M. Liz-Marzan, *J. Phys. Chem. B,* 2005, **109**, 14257.