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# Homogeneous Detection of Human IgG by Gold Nanoparticle Probes

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**Abstract:** A simple, and homogeneous detection system for human IgG based on the optical properties of aggregated gold nanoparticles probes were investigated. When gold nanoparticles with about 13 nm in diameter were modified by goat anti-human IgG, the addition of human IgG could change the absorption of colloidal gold solution, and the absorption intensity at 740 nm depended on the amount of human IgG. The aggregation of gold nanoparticles was also validated using transmission electron microscopy (TEM). A series of experiments were carried out to study the effects of pH value, the reaction temperature, and non-specific adsorption on the assay. A dynamic range of 10-500 μg/3 mL human IgG was observed. The new bioassay could be used for the rapid and homogeneous detection of antibodies in bioanalytical chemistry.

**Key words:** bioassay; human IgG; gold nanoparticles

## 1 Introduction

The immunoassay of protein plays a major role in clinic diagnoses, medicine and related areas[1]. Many methods were reported for the biological measurement of protein or DNA. The radioimmunoassay has revolutionized the bioanalytical chemistry due to its extremely high sensitivity and specificity[2]. However, the special disposal of radioactive waste and the potential radioactive hazards limited its use. Though the enzyme-linked immunosorbent assay (ELISA) has been widely used in biological measurement, the experimental procedure is more complex due to mutli-wishing in immunoassay protocol[3]. Thereby it is desired to develop a simple and homogeneous method for detecting protein.

It is well known that the aggregation of colloidal gold nanoparticles resulted in the red-shift of UV-vis absorption spectra due to the electric dipole-dipole interaction and coupling between the plasmons of neighboring particles in the formed aggregates. And the intensity of the plasmon resonance absorption band in the near-infrared range was pursued for obtaining the details of various targets[4]. Based on the optical properties of functionalized gold nanoparticles, many scientists have recently reported the colorimetry for detecting various targets by using gold nanoparticles modified with different functional molecules[5]. Mirkin *et al* firstly reported an assay using oligonucleotide-functionalized Au nanoparticles that exhibited strong red shifts upon aggregation in the presence of a complementary nucleotide, and the color change in this case provided a test method for determining nucleic acid targets[6-8]. The biotin-avidin system linked to Au nanoparticles was utilized to created a colorimetric assay for kinase inhibitors[9]. Rapid detection of cholera toxin has been demonstrated using lactose-stabilized Au nanoparticles, in which the cholera toxin binds to a lactose derivative and induces nanoparticle aggregation[10]. Lin *et al*[11] demonstrated a colorimetry for detecting $K^+$ ions based on the efficient recognition of $K^+$ ions by 15-crown-5 functionalized gold nanoparticles in aqueous matrix.

In the proposed paper, the aggregation process of gold nanoparticles functionalized with goat-anti-human IgG was investigated by adding human IgG. UV-vis spectra and transmission electron microscopy (TEM) were used to pursue the aggregation process. The influence of pH and temperature on the aggregation process was measured. At optimal conditions, the analytical capabilities were discussed.

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# 2 Experimental

## 2.1 Reagents and apparatus

$HAuCl_4 \cdot 4H_2O$, sodium citrate, $NaBH_4$, concentrated HCl and $HNO_3$ were purchased from Shanghai First Reagent Plant. All reagents were of analytical grade or the best grade commercially available unless otherwise mentioned. 10% bovine serum albumin (BSA) and 2 mg/mL goat-anti-human IgG were obtained from Shanghai Huamei Bioengineer Company. Human IgG was bought from Beijing Jingke Biotechnology Company.

The absorption spectra was recorded with UVIKON-941 spectrophotometer (Kontron Instruments). Transmission electron microscopy (TEM) was performed on JEM-100CXII (80 kV), Japan. One drop of sample solution was dispersed to the holey carbon film on a copper grid for TEM characterization on the surface.

## 2.2 Procedures

2.2.1 Preparation of gold nanoparticles

Colloidal gold seeds were prepared following the method described in Ref.[12]. $HAuCl_4$ (50 mL, 1 mmol/L) was brought to a round-bottomed flask under vigorous stirring. Trisodium citrate(5 mL, 38.8 mmol/L) was rapidly added to the vortex of the boiling $HAuCl_4$ solution, resulting in a color change from pale yellow to burgundy. Boiling was prolonged for 10 min. The heating mantle was then removed, while the stirring was continued for an additional 15 min. The resulting solution of colloidal gold particles was characterized by absorption spectroscopy and TEM. The colloidal gold nanoparticles obtained had a maximum absorption at 520 nm and uniform size (about 13 nm).

2.2.2 Protein-gold nanoparticle conjugates

Pritein-gold nanoparticle conjugates were prepared according to the modification in Ref.[11]. The desired amount of goat-anti-huamn IgG was added to pH-adjusted colloidal Au nanoparticles (10 mL) followed by incubation at room temperature for 1-2 min. Then, 0.1 mL BSA (10%) was injected for embedding the sites not functionalized with goat-anti-human IgG. The conjugate was centrifuged at 10000 rpm for 12 min, and the soft sediment was resuspended in 10% phosphate-buffered BSA solution. The above sample was centrifuged again. The intensity of resulted gold colloid was adjusted to 1.5, and the colloidal Au goat-anti-rabbit conjugates were stored at 4 ℃.

2.2.3 Immunoassay procedures

The immunoassay was carried out by using the typical procedure: The 1 mL solution of functionalized gold nanoparticles was diluted to 3 mL with a 0.3 mol/L NaCl, 10 mmol/L phosphate buffer (pH 7.0). Then, different amount of human IgG solution was added, and the absorption spectra were recorded by using UVIKON-941 spectrophotometer. A series of experiments were conducted to study the effects of pH, the reaction temperature, and non-specific adsorption on the assay.

# 3 Results and Discussion

## 3.1 Immunoaggregation process of functionalized gold nanoparticles

The theories and experimental data have validated that surface plasmon resonances spectra of the gold nanoparticles can be readily dependent on their size, shape, spacing, and dielectric environments[4]. When gold nanoparticles were modified with goat-anti-human IgG, the absorption peak red-shifted from 520 nm to 530 nm, and the intensity decreased. The above results were due to that the modification of gold nanoparticles with protein changed their dielectric environments, and thus, altered their optical properties. When human IgG was added to the phosphate-buffered solution of functionalized gold nanoparticles, a color change from red to purple could be observed within 20 min. The UV-vis absorption spectra of solutions containing gold nanoparticles modified with goat-anti-human IgG and human IgG at different times during the assay are shown in Fig.1. The aggregation of gold nanoparticles led to an increase in absorbance at longer wavelength (600-800 nm). The optical properties of the assays were due to the resonantly exited localized surface plasmon resonances of the gold nanoparticles. The enhanced electronic fields were confined within a small area around the nanoparticles (typically on the order of the particle radius) and decayed approximately exponentially thereafter. As the distance between the nanoparticles decreased, the near-field coupling began to dominate, which led to a strong enhancement of the localized electric field within the interparticle spacing and produced pronounced red shifts of the localized surface plasmon resonances frequency. In our experiments, we found that the largest change in absorption during the aggregation of functionalized gold nanoparticles was at 740 nm. Fig.2 shows the TEM image of functionalized gold nanoparticles before ($t$=0 min, A), and after ($t$=90 min, B) adding human IgG. TEM image of Fig.2(b) shows that the density of the particles in the field of view increased, and the islands of small aggregates appeared. Scheme 1 summarizes the aggregation process of gold nanoparticles modified with goat-anti-human IgG after adding human IgG.

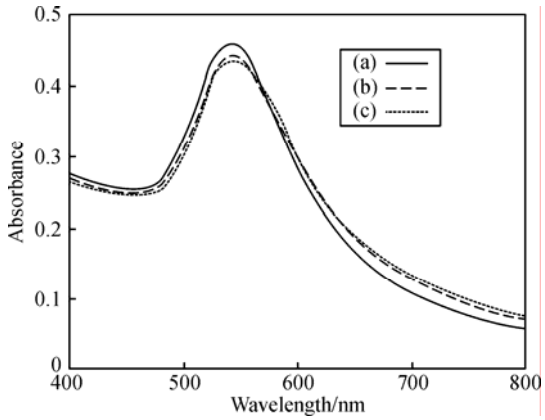



Fig.1 Spectra of the aggregation process were measured at 740 nm from different time: (a) 0 min, (b) 20 min, (3) 40 min

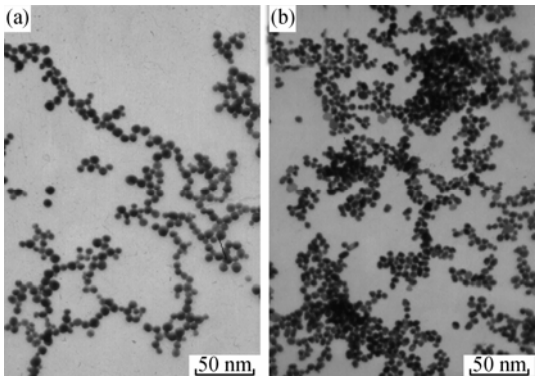
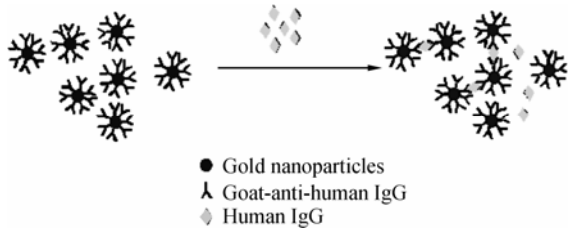



Fig.2 TEM image during the aggregation process at $t$ = 0 (A), and 90 min (B)




Scheme 1 Mechanism of detecting human IgG based on the aggregation of gold nanoparticles modified by goat-anti-human IgG

## 3.2 pH dependence on the immunoaggregation of functionalized gold nanoparticles

The aggregation process of functionalize gold nanoparticles is based on the interaction of antigen-antibody. The reaction between antigen and antibody involves charged species. So the pH volue of reaction system would affect the aggregation process of gold nanoparticles modified with goat-anti-human IgG. When the immunoassay was carried out on the pH range from 4.0 to 9.0, it was shown from Fig.3 that the change of the absorption intensity of functionalized gold nanoparticles at 740 nm is maximal at pH＝7.2. The above result is due to the repulsive electrostatic forces at lower or higher pH[6]. Therefore, all the following experiments were conducted at pH 7.2.

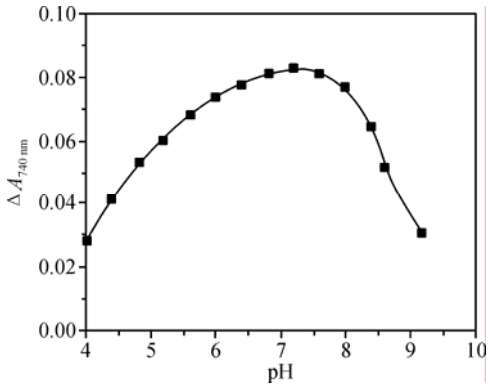



Fig.3 pH dependence of the aggregation rate measured at pH 4.0-9.0. The absorption change of sample containing goat-anti-human IgG-coated nanoparticles and human IgG (150 μg/3 mL) was measured at 740 nm

## 3.3 Temperature dependence on the immunoaggregation process

As shown in Fig.4, the absorption intensity of the aggregation of gold nanoparticles at 740 nm gradually decreases as reaction temperature increases, indicating that the temperature dependence on the aggregation process of functionalized gold nanoparticles is obvious. The aggregation degree of gold nanoparticles decreases as the reaction temperature increases because the increase of reaction temperature would result in the decrease of the stability of the formed aggregates. However, it would take more time to finish the aggregation process of gold nanoparticles if the immunoassay of human IgG carried out at low temperature. Therefore, we chose 30 ℃ for the following assays.

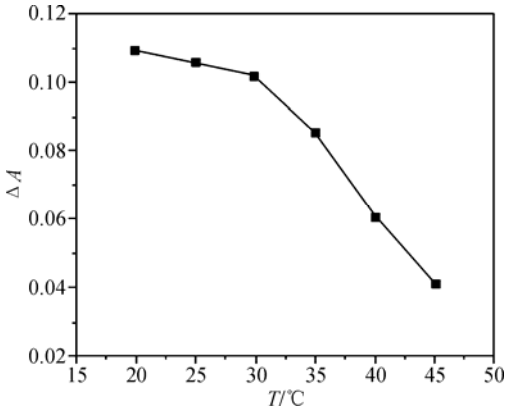



Fig.4 Temperature dependence of the immuno-aggregation of functionalized gold nanoparticles. The assays were carried out in phosphate-buffered solutions (pH 7.2)

## 3.4 Controlled experiments

To validate the selectivity of the aggregation assay, we carried out several controlled experiments, as shown in Fig.5. Curves a, and b show that there was no change of the absorption intensity goat-anti-human IgG and human IgG at 740 nm. Curve c shows that the absorption intensity of functionalized gold nanoparticles is invariable if the target protein was absent. Curves *d-g* show that there is no change for the absorption intensity of functionalized gold nanoparticles at 740 nm after 200 μg

BSA, goat-anti-human IgG, hemoglobin, or myoglobin was added into the reaction system. The non-specific adsorption between four above proteins and goat-anti-human IgG linked to gold nanoparticles resulted in the tiny increase of the absorption intensity compared with that of functionalized gold nanoparticles. However, curve h shows that the increase of the absorption intensity is observed when 150 μg human was added to the reaction system 20 min later. The aggregation of gold nanoparticles modified with goat-anti-human IgG was due to the recognition interaction with the target-human IgG, and the aggregation process would be over after 90 min.

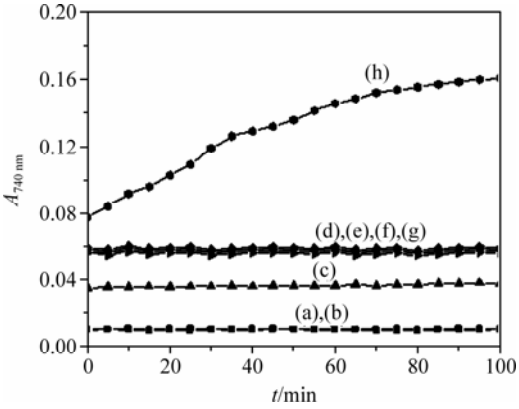



Fig.5 Controlled experiments to validate the selectivity of the aggregation process: (a) 200 μg/mL goat-anti- human IgG; (b) 200 μg/mL human IgG; (c) 200 μg/mL goat-anti-human IgG-coated gold nanoparticles; (d) (c)+200 μg/3 mL BSA; (e) (c)+200 μg/3 mL goat-anti-human IgG; (f) (c)+200 μg/3 mL hemoglobin; (g) (c)+200 μg/3 mL myoglobin; (h) (c)+150 μg/3 mL human IgG. All experiments were carried out in phosphate-buffered solution at pH 7.2 for 100 min

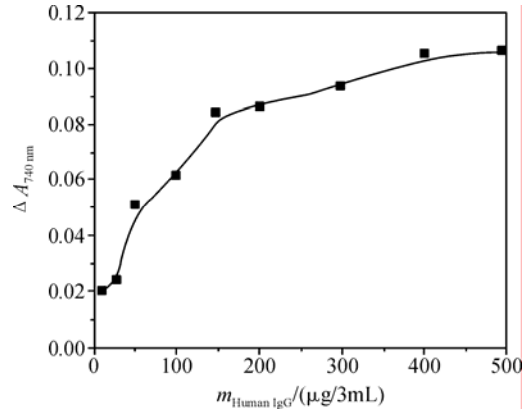



Fig.6 Calibration curve describing the absorption change at 740 nm against human IgG concentration. A dynamic range of 2 orders of magnitude and a limit of detection of 3.3 μg / mL human IgG were observed

### 3.5 Calibration curve of the gold nanoparticle-based assay

The absorption change at 740 nm vs concentration calibration curve for human IgG is shown in Fig.6. A dynamic range of 2 orders of magnitude in concentration was observed when the solution concentrations ranged from 10 to 500 μg/3 mL. When the concentration of human IgG was higher, the excessive target blocked active sites, and inhibited the aggregation process of gold nanoparticles functionalized with goat-anti-human IgG. The relative standard deviation within the plate of 5 samples was 0.9%-3.7 %. The limit of detection of the assay in serum samples was 3.3 μg/mL of human IgG.

## 4 Conclusion

A simple, and homogeneous method for detecting human IgG was developed based on the optical properties of aggregated gold nanoparticles modified with goat-anti-human IgG. The aggregation process of gold nanoparticles modified with goat-anti-human IgG in the presence of human IgG was observed by monitoring the absorption change of the gold nanoparticles upon aggregation at 740 nm. The studies had shown that the immunoassays were successful. The colorimetric method may provide potential for biological assays and clinical diagnoses in the homogeneous system.

## References

[1] E P Diamandis, T K Christopoulos. *Immunoassay*[M]. London: Academic Press, 1996:1-3

[2] T Chard. *An Introduction to Radioimmunoassay and Related Techniques*[M]. Amsterdam: Elsevier Biomedical Press, 1982

[3] R M Lequin. Enzyme Immunoassay (EIA)/Enzyme-linked Immunosorbent Assay (ELISA)7[J]. *Clinical Chemistry*, 2005, 51: 2415-2418

[4] K L Kelly, E Coronado, L L Zhao, *et al*. The Optical Properties of Metal Nanoparticles: the Influence of Size, Shape, and Dielectric Environment[J]. *J. Phys. Chem. B*, 2003, 107: 668-677

[5] M E Stewart, C R Anderton, L B Thompson, *et al*. Nanostructured Plasmonic Sensors[J]. *Chem. Rev.*, 2008, 108: 494-521

[6] R Elghanian, J J Storhoff, R C Mucic, *et al*. Selective Colorimetric Detection of Polynucleotides Based on the Distance-dependent Optical Properties of Gold Nanoparticles[J]. *Science*, 1997, 277: 1078-1081

[7] R A Reynolds, C A Mirkin, R L Letsinger. Homogeneous, Nanoparticle-based Quantitative Colorimetric Detection of Oligonucleotides[J]. *J. Am. Chem. Soc.*, 2000, 122: 3795-3796

[8] M S Han, A K R Lytton-Jean, C A Mirkin. A Gold Nanoparticle Based Approach for Screening Triplex DNA Binders[J]. *J. Am. Chem. Soc.*, 2006, 128: 4954-4955

[9] Z X Wang, R Levy, D G Fernig, *et al*. Kinase-catalyzed Modification of Gold Nanoparticles: a New Approach to Colorimetric Kinase Activity Screening[J]. *J. Am. Chem. Soc.*, 2006, 128: 2214-2215

[10] C L Schofield, R A Field, D A Russell. Glyconanoparticles for the Colorimetric Detection of Cholera Toxin[J]. *Anal. Chem.*, 2007, 79: 1356-1361

[11] S Y Lin, S W Liu, C M Lin, *et al*. Recognition of Potassium Ion in Water by 15-Crown-5 Functionalized Gold Nanoparticles[J]. *Anal. Chem.*, 2002, 74: 330-335

[12] K G Grabar, R G Freeman, M B Hommer, *et al*. Preparation and Characterization of Au Colloid Monolayers[J]. *Anal. Chem.*, 1995, 67: 735-743