Analytical Methods

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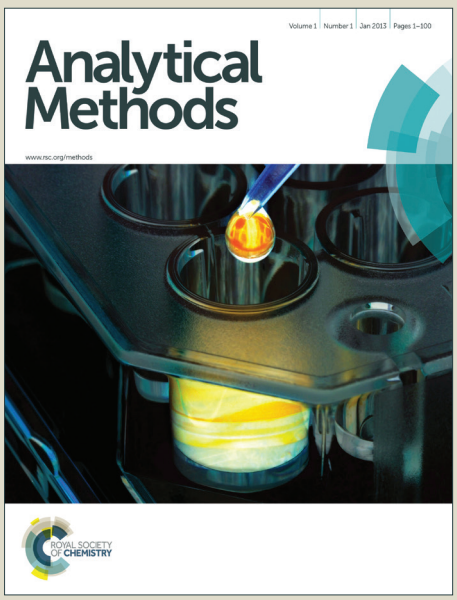

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ARTICLE

Visual Detection of 8-OHdG Based on the Aggregation of Gold Nanoparticles Capped with Anti-8-OHdG Antibody

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Gold nanoparticles (AuNPs) were stably capped with anti-8-OHdG polyclonal antibody. When 8-OHdG was added, the antibody-capped AuNPs would aggregate for the specific interaction of antibody and antigen. The maximum absorption wavelength of antibody-capped AuNPs red shifts gradually and the solution color changes from red to blue, with the increasing concentration of 8-OHdG. Based on that, highly selective determination of 8-OHdG is set up, with the detecting range of 0.25-5.00 μg·mL^{-1} and LOD of 25 ng·mL^{-1}. The method is simple, rapid and easy to be operated. Semi-quantitative determination can also be achieved by visual observation of the solution color change without any apparatus.

Introduction

8-hydroxydeoxyguanosine (8-OHdG) is a remarkable biomarker of DNA oxidative damage. Basically, DNA oxidative damage occurs when reactive oxygen species (ROS) is accumulated excessively. Because of the low electrode potential, the eighth carbon atom of base G in DNA molecules will be hydroxylated when attacked by ROS. And then, 8-OHdG is produced[1].

8-OHdG in serum or urine is highly associated with human diseases. A certain disease can be effectively predicted by monitoring the amount of 8-OHdG. For instance, aflatoxin and microcystin are two of the most important factors that can induce liver cancer. When exposed to the two toxins, plenty of ROS will be produced and oxidative damage to DNA will happen, resulting in the increasing 8-OHdG level in human bodies. Primary hepatic carcinoma is strongly related to the accumulation of 8-OHdG in hepatic cells in the long term[2,3]. Besides, 8-OHdG is an effective biomarker of cerebral injury. The degree of cerebral injury can be estimated by monitoring 8-OHdG in urine continuingly[4]. 8-OHdG in urine could also serve as the marker of oxidative stress for lung cancer patients[5]. Moreover, 8-OHdG level is highly connected with breast cancer[6], diabetes[7], cardiomyopathy[8], atherosclerosis[9]. So, it is definitely meaningful to establish a detection method for 8-OHdG, which is simple, rapid and effective.

At present, the reported methods for detecting 8-OHdG include HPLC-EDC[10], HPLC-MS[11], CE[12,13], Resonance Rayleigh Scattering spectra[14], electrochemical performance[15], ELISA[16], quartz crystal microbalance hybrid sensor system[17], and so on. However, there are still disadvantages. For example, expensive instruments are needed; operation is complicated; determination is time-consuming; or the selectivity is not satisfactory. Thus, it is necessary to develop a simple, rapid and visual method to detect 8-OHdG.

Attributed to the distinctive properties of optics, electrics and color change, gold nanoparticles (AuNPs) have been widely applied in the fields of biological medicine[18], monitoring of toxins in environments[19], determination of tumor biomarkers[20] and so forth. The advantages of AuNPs, as the biological probe, are simple preparation, low toxicity, excellent biological compatibility and easy to be modified on the surface. The antigens, such as vascular endothelial growth factor (VEGF)[21] and C-reactive protein[22], can be effectively detected, when combining the particular properties of AuNPs and the specific interaction of antibody-antigen.

In this contribution, anti-8-OHdG polyclonal antibody was stably capped on the surface of AuNPs first. When 8-OHdG was added, AuNPs capped with antibodies would aggregate for the specific interaction between 8-OHdG and its antibody, resulting in the color change of solution and the redshift of the absorption spectra. Based on the phenomenon and the absorption signal, the visual and quantitative determination of 8-OHdG was achieved, with the detection range of 0.25-5.00 μg·mL^{-1} and the LOD (limit of detection) of 25 ng·mL^{-1}. The advantages of this absorption method are listed as follows. First of all, the detecting principle is easy to be understood and operation is also simple to be done. Secondly, semi-quantitative determination can even be achieved by visual observation of the solution color change without any apparatus. Finally, our method is based on the specific interaction between antibody and antigen. So the selectivity is really good.

Experimental

Materials and reagents. $HAuCl_4 \cdot 4H_2O$ was purchased from Sinopharm chemical reagent limited company (Shanghai, China). $Na_3C_6H_5O_7 \cdot 2H_2O$ was bought from Kelong chemical reagent limited company (Chengdu, China). 8-OHdG was purchased from Cayman chemical reagent limited company (Michigan, USA). 8-OHdG was dissolved in deoxidized DMSO (oxygen free). 2.0 mg·mL^{-1} of stock

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solution was made and stored at 4°C. 20 μg·mL^{-1} of 8-OHdG working solution was made by diluting 10 μL 8-OHdG stock solution in 200 μL 0.1 M of HCl solution and 790 μL PBS buffer (0.01M, pH7.2). The pH of HCl can be neutralized by PBS buffer when the volume ratio is 1:3.5, based on the instruction of 8-OHdG. 8-OHdG working solution could only be prepared when used, and saved for one day at most. 100 μg·mL^{-1} of anti-8-OHdG antibody (J-1, sc-139586), a rabbit polyclonal antibody, was bought from Santa Cruz Biotechnology, Inc. (Texas, USA). 1 μg·mL^{-1} of the working solution was diluted with PBS buffer (0.01M, pH7.2) and stored at 4°C. 10.0% of BSA stock solution was used to block free sites on the surface of AuNPs, and 1.0% of NaN_3 stock solution was as a preservative to store AuNPs capped with antibody. 10.0% of NaCl solution was used to test the stability of AuNPs. Britton-Robinson (BR) buffer was to adjust the pH of the system. All the reagents were no need of further purification and the water used was doubly distilled (18.2MΩ).

Apparatus. Absorption spectra were measured with TU-1901 double-beam UV-Vis spectrophotometer (Beijing, China). DF-101B thermo and magnetic stirrer (Zhejiang, China) was used when AuNPs were made. TEM images were obtained with TECNAI10 transmission electron microscopy (Philips, Holland). R404A refrigerated centrifuge (Eppendorf, German) was used to spin down AuNPs. MX-F fixed vortex mixer (Beijing, China) was used to mix solutions.

Synthesis of AuNPs. AuNPs was synthesized according to the reported reference of reducing $HAuCl_4$ by trisodium citrate[23]. 96 mL doubly distilled water was added into 250 mL conical flask and boiled with stirring. 4 mL of 1% $HAuCl_4$ solution was then added in the boiling water. At last, 2 mL of 5% trisodium citrate solution was quickly dropped with vigorous and continuous stirring. The color of the mixed solution turned from yellow to wine red within three minutes. The solution was kept boiling until the wine color was stable and not to be changed, and then cooled to room temperature with nonstop stirring. The obtained AuNPs was stored at 4°C for further use. The concentration of AuNPs can be calculated according to Lambert-Beer law, with the extinction coefficient of 2.7×10^8 $M^{-1}\cdot cm^{-1}$ at 520 nm for 13-nm gold nanoparticles[24]. The size of synthesized AuNPs was 13.1 ± 1.4 nm based on the TEM image and the absorption intensity was 0.611 after five-fold dilution, when detected in a 1-cm absorption cell. So the original concentration of AuNPs solution was 11.3 nM.

Optimization of pH, buffer and antibody amount when AuNPs were capped. In order to determine 8-OHdG, the anti-8-OHdG antibody should be capped on the surface of AuNPs. The best pH, buffer and antibody amount should be optimized in this process. For pH optimization, 100 μL of AuNPs solution, 30 μL of doubly distilled water, 30 μL of BR buffer with certain pH, and 120 μL of 1 μg·mL^{-1} anti-8-OHdG antibody were added in EP tube, respectively. The mixed solution was vortexed and incubated at room temperature for 30 min. 20 μL of 10.0% NaCl solution was added to the mixture. Then we chose the best pH condition, based on whether AuNPs aggregated or not in two hours, for well-capped AuNPs have the resistance against high concentration of NaCl solution. The best amounts of BR buffer and antibody were also optimized with the same method here.

Synthesis of antibody-capped AuNPs. Under optimal conditions, we synthesized the antibody-capped AuNPs. 10.0 mL of AuNPs solution, 1.5 mL of doubly distilled water, and 4.5 mL of BR buffer with pH 7.24 were mixed first in a 100 mL beaker. 14.0 mL of 1 μg·mL^{-1} anti-8-OHdG antibody was added, mixed and incubated at room temperature for 30 min. Then, BSA was added with the final concentration of 1.0% to block free sites on the surface of antibody-capped AuNPs. The mixed solution was then centrifuged at 10000 rpm and 4°C for 30 min. The pellets were washed twice with PBS buffer (0.01M, pH7.2). At last, the well-capped AuNPs were resuspended in 10.0 mL of PBS buffer (0.01M, pH7.2) containing 1.0% BSA and 0.02% NaN_3 and stored at 4°C for further use[25].

General Procedures of 8-OHdG Determination. 100 μL of well-capped AuNPs solution was added into a 1.5-mL EP tube, and then the appropriate amount of 8-OHdG working solution was added in the same tube. The doubly distilled water was added to the total volume of 400 μL, and the mixed solution was vortexed thoroughly. The color change was recorded with camera, and the absorption spectra were measured over the wavelength range of 400 nm to 700 nm. The selectivity experiment was carried out in the same way, just adding foreign substances instead of 8-OHdG.

Results and Discussion

Characteristics of Absorption spectra. It is shown from the absorption spectra (Fig. 1) that the characteristic peak of synthesized AuNPs is located at 516 nm (Curve 1). And the peak of AuNPs capped with anti-8-OHdG antibody is located at 528 nm with the absorption intensity decreased a little bit (Curve 2). It is reported that macromolecules, such as protein and DNA, could adsorb on the surface of nanoparticles through electrostatic and/or hydrogen-bond interactions[26,27]. And the micro surroundings of nanoparticles are different so that the spectra changed[28,29]. That is

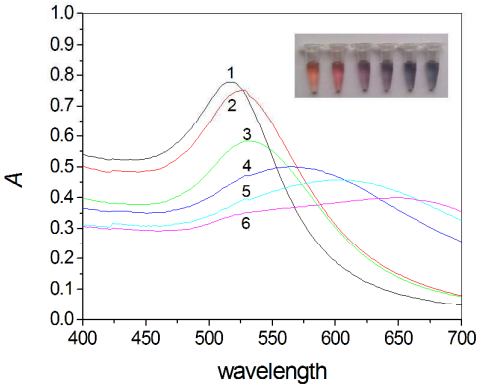

Fig.1 Absorption Spectra of the interaction between antibody-capped AuNPs and 8-OHdG (Curve 1, AuNPs only; Curve 2, antibody-capped AuNPs; Curve 3-6, interaction between antibody-capped AuNPs and 8-OHdG). Inset: color change corresponding to Curve 1-6. Concentrations: 8-OHdG (Curve 1-6, μg·mL^{-1}), 0, 0, 0.25, 1.50, 3.50, 5.00.

why the characteristic peak redshifts when AnNPs are capped with antibody. It is further proved that anti-8-OHdG antibody has been successfully capped on the surface of AuNPs by the change of the characteristic peak[30,31]. The decrease of the absorption intensity was caused by the inevitable loss over the process of the operations, such as centrifugation and resuspension, when antibody-capped AuNPs were synthesized.

Curves 3 to 6 represent the situation when different concentrations of 8-OHdG were added in the solution of antibody-capped AuNPs. It is shown that the characteristic absorption peak redshifts gradually with the increasing concentration of 8-OHdG. The inset picture shows the color change of the mixture according to Curve 1 to 6. We can see that the color turns from red to purple and then to blue with the increase of 8-OHdG. Based on that, 8-OHdG can be detected visually and semi-quantitatively.

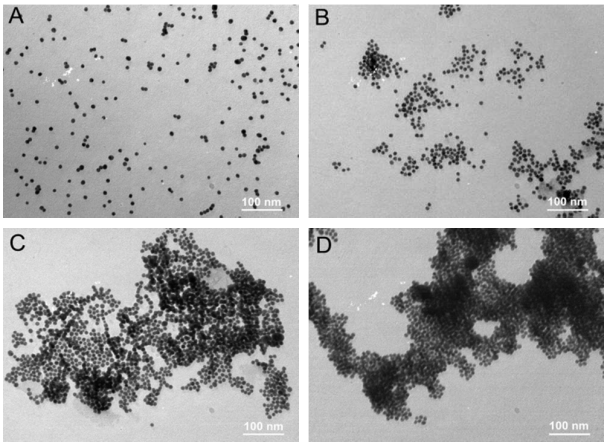

Fig.2 TEM images of the interaction between antibody-capped AuNPs and 8-OHdG (A, antibody-capped AuNPs; B-D, interaction between antibody-capped AuNPs and 8-OHdG). Concentrations: 8-OHdG (A-D, μg·mL^{-1}), 0, 0.25, 1.50, 3.50.

It can be seen from the TEM images that antibody-capped AuNPs are singly dispersed (Fig. 2A). While interacting with 8-OHdG, AuNPs will aggregate and the aggregation gets stronger with the increasing concentration of 8-OHdG (Fig. 2B-D). So the redshift of wavelength and the color change were caused by the aggregation of antibody-capped AuNPs.

Mechanism of 8-OHdG determination. It has been reported that macromolecules, like proteins, could be strongly adsorbed on the surface of nanoparticles. In our experiment, AuNPs are hydrophobic colloid with negative charge. Anti-8-OHdG is positive charged at certain pH condition. So the anti-8-OHdG antibody can be stably capped on the surface of AuNPs through the electrostatic interactions. The anti-8-OHdG antibody we used was a rabbit polyclonal antibody. A polyclonal antibody represents a collection of antibodies produced from different B cells which can recognize multiple epitopes on the same antigen. In other words, 8-OHdG, as antigen, will bind more than one polyclonal antibody molecule on multiple epitopes. When 8-OHdG was added into the solution of antibody-capped AuNPs, the distance between nanoparticles will be

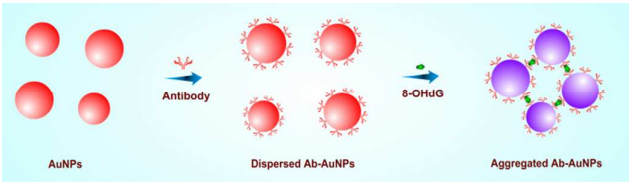

Fig.3 Schematic diagram of 8-OHdG determination

extremely shortened attributed to the specific interaction of the antibody and antigen. Thus, the nanoparticles aggregate (shown in Fig. 3). The more amount of 8-OHdG, the more obvious aggregation will be, within the given concentration range. The color of the dispersed nanoparticles is red. And, it will turn to blue when aggregating. Meanwhile, the absorption spectra red shift gradually. Based on the relationship between the maximum absorption wavelength and the 8-OHdG concentration, the quantitative determination of 8-OHdG could be set up.

Optimization of pH and the buffer amount when AuNPs were capped with antibody. The proper pH is needed when we make antibody-capped AuNPs. The well capped AuNPs with macromolecules are able to resist the high-concentration salt to avoid aggregation. So we optimized pH based on this principle. AuNPs and anti-8-OHdG antibody were mixed and incubating for 30 min at different pH, which was provided by BR buffer. After that, NaCl was added into the mixture. AuNPs would not aggregate or the absorption spectra would not redshift, if AuNPs were stably capped with antibodies. It is shown in Fig. 4 that, with adding NaCl, AuNPs aggregate and the characteristic peak redshifts to 568 nm or even 594 nm, when pH is less than 5.72, indicating that AuNPs could not be well capped by anti-8-OHdG antibody under low pH conditions. Whereas, AuNPs could be well capped and the characteristic peak keep at 528 nm when pH was over the range from 5.72 to 9.62. So we choose a neutral pH of 7.24.

Meanwhile, the optimal amount of BR buffer also needs to be tested because AuNPs will be unstable due to high ionic strength if buffer is too much; on the other hand, it will not be buffered enough if buffer amount is insufficient. So we optimized the BR buffer amount. It was found that there was no influence on the capping of AuNPs, when the volume percentage of BR buffer was between 2.5%-25% (v/v). Then, we adopted 15% of buffer volume.

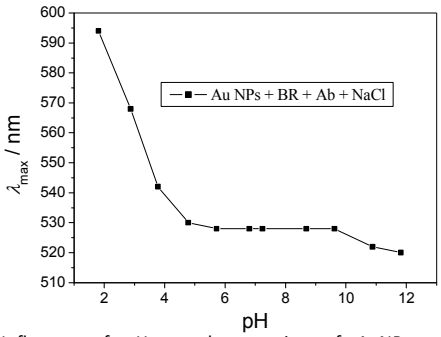

Fig. 4 Influence of pH on the capping of AuNPs with antibody. Concentrations: AuNPs, 3.77 nM; anti-8-OHdG antibody, 0.40 μg·mL^{-1}; NaCl, 0.67%; pH, 1.81, 2.87, 3.78, 4.78, 5.72, 6.80, 7.24, 8.69, 9.62, 10.88, 11.82.

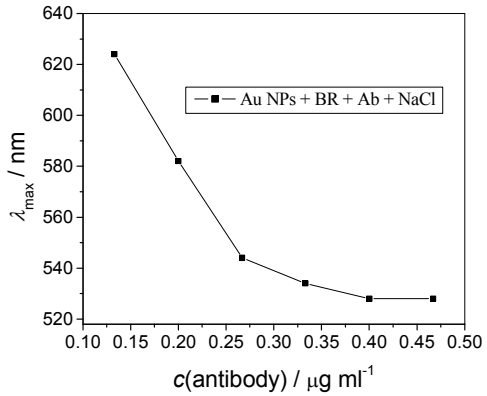

Fig. 5 Influence of antibody concentration on the capping of AuNPs. Concentrations: AuNPs, 3.77 nM; pH, 7.24; NaCl, 0.67%; anti-8-OHdG (μg·mL^{-1}), 0.13, 0.20, 0.27, 0.33, 0.40, 0.47.

Optimization of antibody amount when AuNPs were capped. The optimal amount of antibody should also be confirmed. It is shown in Fig. 5 that the wavelength would red shift with the addition of NaCl, when the concentration of antibody was low, which means that the antibody amount was not abundant enough to be adsorbed on the surface of AuNPs, resulting in the aggregation with high salt. In contrast, AuNPs could well resist against high salt when the concentration of antibody is more than 0.40 μg·mL^{-1}. So, 0.40 μg·mL^{-1} is the optimal amount of anti-8-OHdG antibody. Given the loss in the process of preparation, the actual amount of antibody is 10-20% more than the optimal one.

Selectivity for 8-OHdG determination. AuNPs were capped with antibody under the optimal conditions, and then it was used to detect 8-OHdG. Meanwhile, we investigated the selectivity of this method. It was found that the AuNPs would aggregate, along with the color change of the mixture and the redshift of absorption wavelength, when 8-OHdG was added into the detecting system.

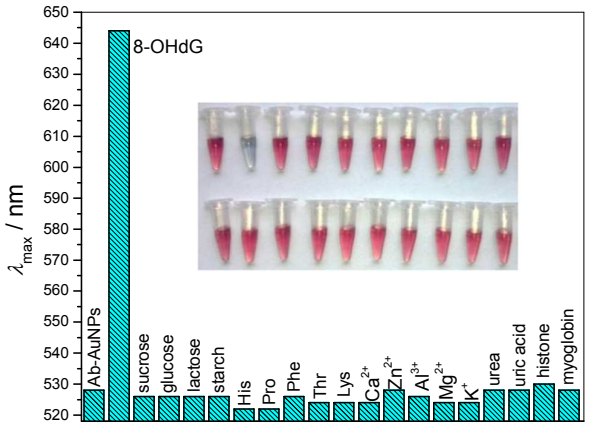

Fig. 6 Selectivity for the 8-OHdG determination. Concentrations (μg·mL^{-1}): 8-OHdG, 5.00; sucrose, 410.8; glucose, 216.2; lactose, 410.8; starch, 125.0; His, 155.1; Pro, 115.1; Phe, 165.1; Thr, 119.1; Lys, 146.2; Ca^{2+}, 40.1; Zn^{2+}, 65.4; Al^{3+}, 27.0; Mg^{2+}, 24.3; K^{+}, 195.5; urea, 240.2; uric acid, 168.1; histone, 100; myoglobin, 33.

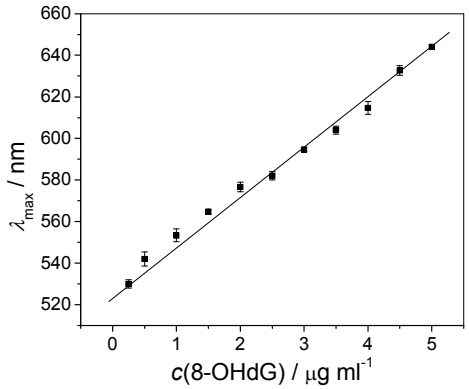

Fig. 7 Calibration curve for 8-OHdG determination. The error bars represent the standard deviation from three independent measurements. Concentrations: 8-OHdG (μg·mL^{-1}), 0.25, 0.50, 1.00, 1.50, 2.00, 2.50, 3.00, 3.50, 4.00, 4.50, 5.00.

Whereas other foreign substances would not affect the detection, such as saccharides, amino acids, metal ions, proteins, urea, uric acid and so forth (shown in Fig. 6), indicating the high selectivity for the determination of 8-OHdG. It is absolutely the great advantage based on the interaction between antibody and antigen.

Determination of 8-OHdG Samples. In this contribution, we use antibody-capped AuNPs to determine the concentration of 8-OHdG. The calibration curve is shown in Fig.7. There is a linear relationship between the maximum absorption wavelength and the concentration of 8-OHdG over the range of 0.25-5.00 μg·mL^{-1}, with the linear regression equation, λ_{max}= 528.48 + 22.54*c*. The limit of determination is 25 ng·mL^{-1} and the correlation coefficient is 0.9965. It can be calculated from the linear equation that λ_{max} is 528.48 nm when the concentration of 8-OHdG is 0 μg·mL^{-1}, which is in accordance with the maximum wavelength of the antibody-capped AuNPs we determined (528 nm), indicating the accuracy of the calibration curve. What is more, the color of solution turns to light purple and then to dark purple when 8-OHdG concentration is between 0.25 and 2.00 μg·mL^{-1}. And the color turns to blue when the concentration is higher than 2.00 μg·mL^{-1}. So we can semi-quantitatively analyse the 8-OHdG amount from the color change observed by naked eyes without any apparatus. This is definitely another advantage of this method.

We determined the 8-OHdG concentration in two samples and the result is shown in Table 1. We can see that the detection recovery is between 91.4-99.9% and RSD is 1.46-2.66%, which is less than 3.0%, for the parallel determinations. So the quantitative determination of 8-OHdG can be realized with this method.

Table 1 Determination results for 8-OHdG samples (n=10)

No.	Added (μg·ml^{-1})	Main Additives	Found (μg·ml^{-1})	Recovery (%)	RSD (%)
1	3.00	urea, Mg^{2+}, glucose, His	2.93	96.9-99.9	1.46
2	2.50	uric acid, K^{+}, sucrose, Thr	2.32	91.4-98.5	2.66

Concentrations (μg·mL^{-1}): urea, 240.2; Mg^{2+}, 24.3; glucose, 216.2; His, 155.1; uric acid, 168.1; K^{+}, 195.5; sucrose, 410.8; Thr, 119.1.

Conclusions

In this contribution, anti-8-OHdG polyclonal antibody was stably capped on the surface of AuNPs, which could serve as a probe for the determination of 8-OHdG. Meanwhile, the pH and the amount of BR buffer and antibody were optimized in this process. Finally, the visual, rapid and semi-quantitative detection of 8-OHdG was carried out through the color change of antibody-capped AuNPs solution, and accurately quantitative determination was realized based on the linear relationship between the maximum absorption wavelength and the concentration of 8-OHdG, with the advantages of easy operation, excellent selectivity and rapid detection. Besides, the sensitivity of this method will be greatly improved, if we combine the solid phase extraction (SPE)[10] and resonance light scattering (RLS) technologies, which is going on recently. Other antigens could also be monitored using the principle we mentioned here.

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Abstract

Gold nanoparticles (AuNPs) were stably capped with anti-8-OHdG polyclonal antibody. When 8-OHdG was added, the antibody-capped AuNPs would aggregate for the specific interaction of antibody and antigen. The maximum absorption wavelength of antibody-capped AuNPs red shifts gradually and the solution color changes from red to blue, with the increasing concentration of 8-OHdG. Based on that, highly selective determination of 8-OHdG is set up, with the detecting range of 0.25-5.00 $\mu g \cdot mL^{-1}$ and LOD of 25 $ng \cdot mL^{-1}$. The method is simple, rapid and easy to be operated. Semi-quantitative determination can also be achieved by visual observation of the solution color change without any apparatus.

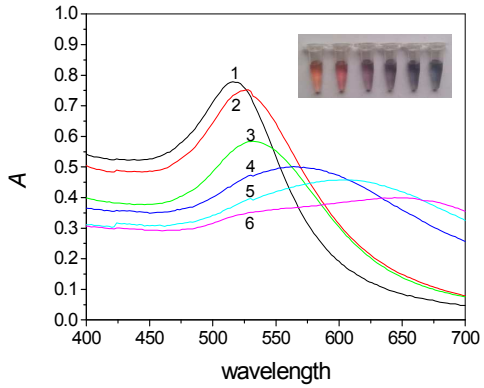

Fig.1 Absorption Spectra of the interaction between antibody-capped AuNPs and 8-OHdG (Curve 1, AuNPs only; Curve 2, antibody-capped AuNPs; Curve 3-6, interaction between antibody-capped AuNPs and 8-OHdG). Inset: color change corresponding to Curve 1-6. Concentrations: AuNPs, 2.82 nM; 8-OHdG (Curve 1-6, $\mu g \cdot mL^{-1}$), 0, 0, 0.25, 1.50, 3.50, 5.00.