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ARTICLE TYPE

# Highly sensitive and rapid visual detection of ricin using unmodified gold nanoparticle probe†

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Herein, a sensitive and selective colorimetric biosensor for the detection of ricin was demonatrated with a 40 - mer ricin-binding aptamer (RBA) as recognition element and unmodified gold nanoparticles (AuNPs) as probe. The sensitivity of the assay was greatly improved after optimizing several key parameters such as the amount of aptamer adsorbed on AuNPs, the concentration of NaCl, and the reaction time after adding NaCl. The linear range for the current analytical system was from 0.31 nM to 11.55 nM. The corresponding limit of detection (LOD) was 0.31 nM. Some different proteins such as thrombin (Th), Horseradish Peroxidase (HRP), lysozyme (Lys), glucose oxidase ($GO_X$), and bovine albumin (BSA) showed no or just a little interference in the determination of ricin. This colorimetric aptasensor is superior to the other conventional methods owing to its simplicity, low cost, high sensitivity and detection with the naked eye, which can be used in real samples.

## 1.Introduction

Ricin is a highly toxic protein which is isolated from the seeds of castor bean Ricinus communis.[1] The ricin toxin consists of two chains (A and B) approximately of equal size, connected by a disulfide bond. Ricin A chain is toxic to cells which inhibits the protein synthesis, thus leading to cell death. Ricin B chain is essential for binding to galactosyl residues on the cell surface and it is responsible for delivering the ricin A chain into the cell. Ricin has been considered as a bio-warfare agent and its toxicity, easy availability of raw materials is a major concern in terrorism. The best-known use of ricin might have been the 1978 assassination of Georgi Markov, a Bulgarian dissident.[2-3] Toxic effects of ricin in animals and humans can be caused by inhalation, oral or intravenous injection. The $LD_{50}$ is 5–10μg/kg (mice, injection). Hence the detection of ricin has become more urgent.

Various technologies have been developed for ricin detection. One kind of these technologies is instrumental analysis, including Fourier transform near-infrared reflectance spectroscopy assay, [4] capillary electrophoresis assay,[5] surface-enhanced Raman spectroscopy.[6] Beside the methods mentioned above, immunoassaywas widely used in the detection of ricin, such as immune-polymerase chain reaction assay, [7] enayme-linked immunosorbent assay (ELISA), [8-10] immunochromatography assay[11-12] and galactose-functionalized magnetic iron-oxide nanoparticles immunoassay.[13] Although, these assays are effective for ricin detection, most of these analytical methods are time-consuming or requiring excellent performances, and tedious procedures for sample pretreatment or preconcentration.

Compared with other analytical assays, colorimetric aptasensors using modified AuNPs have been attracted more and more attention due to their high sensitivity. However, the modification of DNA onto the AuNPs often requires the separation procedure of the modified AuNPs. Apparently, these steps are rather time-consuming and corresponding highly cost. Hence, the development of unmodified AuNPs-based colorimetric biosensors to simplify the detection process would be important and attractive. To date, many researchers extended the target to various analytes, such as thrombin,[14-15] oxytetracycline,[16] ochratoxin A,[17] dopamine,[18] and SDM.[19] They used functional DNA (aptamers) as recognition elements of those assays.

As a new class of single-stranded DNA/RNA molecules, aptamers are selected in vitro by the systematic evolution of the ligand by the exponential enrichment (SELEX) process from random-sequence nucleic acid libraries. Aptamers are short nucleic acid ligands which can bind a wide range of target molecules including proteins, drugs, small molecules, inorganic ions and even cells with high affinity and specificity. [20-25]

In this study, we report a colorimetric detection method of ricin using ricin binding aptamer (RBA) as recognition element and unmodified gold nanoparticles as probe. RBA could be adsorbed onto the surface of AuNPs and protect the AuNPs from NaCl-induced aggregation. However, in the presence of ricin, RBA would undergo a conformation variation, and lose the ability to

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protect AuNPs, thus resulting in NaCl-induced aggregation. The detection could be realized by monitoring the color change of the AuNPs even with naked eyes.

## 2. Materials and methods

### 2.1. Reagent and chemicals

Ricin was purchased from Beijing Hapten and Protein Biomedical Institute (Beijing, china). The sequence of ricin binding aptamer (RBA) was 5'-ACACCCACCGCAGGCAGACGCAACGCCTCGGAGACTAG CC-3'.[26] The ssDNA oligonucleotides were synthesized by Shanghai Sangon Biotechnology Co. Ltd. (Shanghai, China) and the lyophilized powder was dissolved in distilled water and before use it was stored at 4 ℃. The concentration of the oligonucleotide was determined by measuring the UV absorbance at 260 nm. Chloroauric acid ($HAuCl_4$•$4H_2O$) was obtained from Shanghai Chemical Reagent Company (Shanghai, China). Thrombin (Th), horseradish peroxidase (HRP), lysozyme (Lys), glucose oxidase (Gox), and bovine albumin (BSA) was purchased from Sigma-Aldrich Chemical Co (Milwaukee, WI, USA). $C_6H_5Na_3O_7$, NaCl, $Na_2HPO_4 \cdot 12H_2O$ and $NaH_2PO_4 \cdot 2H_2O$ were obtained from Beijing Chemical Reagent Company. All of the reagents were analytic grade and used as received. Ultrapure water obtained from a Millipore water purification system ( ≥18 MΩ, Milli-Q, Millipore) was used in all runs.

### 2.2. Instrumentation

The ultraviolet-visible (UV-vis) absorption spectra were recorded on a Cary 50 Scan UV-vis spectrophotometer (Varian, USA) at room temperature. Transmission electron microscopy (TEM) measurements were made on a Hitachi H-8100 transmission electron microscope operated at an accelerating voltage of 100 kV.The hydrodynamic size was measured on Malvern Zetasizer Nanoseries at 25 ℃.

### 2.3. Synthesis of the citrate-protected AuNPs

AuNPs were synthesized using the classical citrate reduction method.[27] Briefly, colloidal AuNPs with an average diameter of 13 nm were prepared by rapidly injecting a sodium citrate solution (10 mL, 38.8 mM) into a boiling aqueous solution of $HAuCl_4$•$4H_2O$ (100 mL, 1 mM) with vigorous stirring. After boiling for 30 min, the reaction flask was removed from the heat to allow the reaction solution to cool at room temperature. The concentration of the AuNPs was about 10 nM, which was determined according to Beer's law by using the extinction coefficient of $2.78*10^8 M^{-1} cm^{-1}$ for 13 nm AuNPs in diameter at 520 nm. [28]

### 2.4. Detection of ricin using colorimetric biosensing method

A typical colorimetric analysis was realized as following procedure: first, 100 μL of 13 nm AuNPs was mixed with 15 μL of 1 μM RBA. Second, 15 μL ricin with appropriate concentration in PBS was added to the AuNPs/RBA solution. The solutions were allowed to react for 5 min and then 50 μL of 0.5 M NaCl was added to produce color change. After the solution was equilibrated for 5 min, the resulting solution was transferred to a quartz cuvette. The UV–vis absorption spectrum was measured over the wavelength ranging from 400 nm to 750 nm. All assays were herein performed at room temperature.

### 2.5. Optimization of key parameters

To improve the sensitivity of the assay for ricin, key parameters in the procedure was optimized as following: (1) the aptamer concentration was investigated from 0.5 to 5μM; (2) the NaCl concentration was investigated from 0.1 to 1 M. (3) the reaction time after adding NaCl was investigated from 1 min to 30 min.

### 2.6. Treatment of milk powder and Pepsi Cola

Milk powder samples were prepared following a previous method with a minor modification.[29] Briefly, 5.0 mg of milk powder was placed in a 7 mL centrifuge tube, and 1.5 mL of 2 M trichloroacetic acid was introduced. After ultrasonication for 10 minutes, the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatants were adjusted to pH 7.0 with NaOH solution, filtered with 0.22 μm membrane and diluted 25 times before use. Pepsi Cola was diluted 25 times with PBS before use.

## 3. Results and discussion

### 3.1. Mechanism of the colorimetric detection of ricin

In this study, the AuNPs solution was stabilized by the citrate anions as their repulsion prevented the AuNPs from aggregating. With the addition of salt such as NaCl, it would neutralize the negative charge of citrate and lead to the AuNPs aggregation. However, it has been reported that ssDNA with a random coil structure could be easily adsorbed onto the surface of AuNPs through the coordination interaction between the nitrogen atoms of the exposed bases and the AuNPs, thus increasing negative charges to the AuNPs and preventing AuNPs against the NaCl-induced aggregation.[30-31] Whereas, in the presence of the target molecule, the relative ssDNA would undergo a conformation variation, and lose the ability to protect AuNPs, thus resulting in NaCl-induced aggregation. Based on the facts mentioned above, the mechanism of the ricin biosensor is shown in Fig. 1. As can be seen in Fig. 1, ricin could specifically bind to the ricin-binding aptamer (RBA) and induced the RBA conformation variation. So as expected, the aggregation of AuNPs occurred due to the addition of ricin, with the color changing from red to blue.

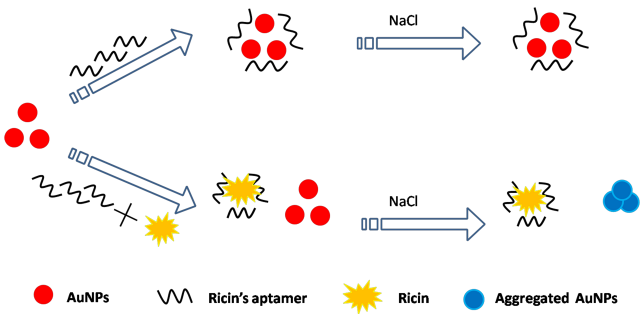


**Fig.1** The mechanism of the colorimetric detection of ricin utilizing RBA and unmodified AuNPs.

### 3.2. Spectral characteristics

Fig. 2A shows UV–vis spectra of AuNPs solution under different experimental conditions. As was shown in Fig. 2A, in the presence of NaCl, the absorption peak of AuNPs red shifted and broadened. The peak at 520 nm decreased and a new peak at about 670 nm appeared, implying that the AuNPs were aggregated. However, with the addition of RBA, it could be seen

in Fig. 2A that there was only one absorption peak at about 520 nm, indicating that the AuNPs were still dispersed. This was owing to RBA with a random coil structure was able to be adsorbed onto the AuNPs and protect AuNPs from salt-induced aggregation. Next, upon the addition of RBA and ricin, it could be apparently observed in Fig. 2A that the adsorption band of AuNPs shifted with a new peak appearing at about 670 nm, moreover, the absorption at 670 nm increased with the increase of the concentration of ricin (Fig. 2A), indicating the aggregation of AuNPs. Fig. 2B shows a visible color change corresponding to Fig. 2A. Fig. 2C and D utilized the transmission electron microscope (TEM) technology to characterize the morphology change of AuNPs. Fig. 2C apparently showed that the AuNPs was still dispersed after the addition of NaCl in the presence of RBA. Dynamic light scattering (DLS) measurements show that the hydrodynamic diameter of AuNPs is 41.19 nm (ESI, Fig.S1 A†). Fig. 2D showed that the AuNPs aggregated after the addition of NaCl in the presence of both ricin and RBA. Dynamic light scattering (DLS) measurements show that the hydrodynamic diameter of AuNPs is 816 nm (ESI, Fig.S1 B†). All these results were in line with the mechanism of the colorimetric detection.

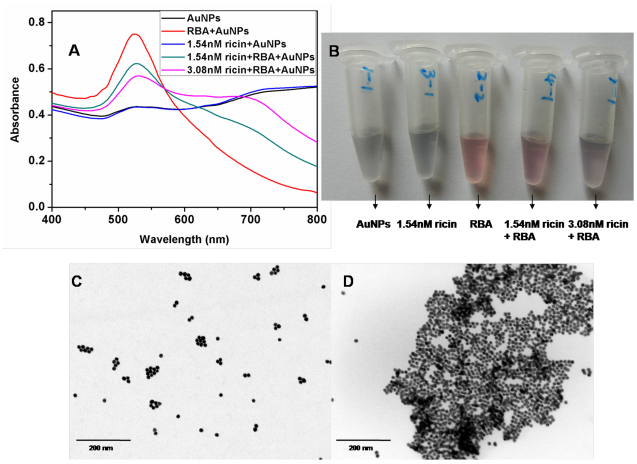


**Fig.2** (A) UV–vis absorption spectra of AuNPs in the presence of 0.138M NaCl under different experimental conditions, cRBA = 83 nM, cAuNPs = 2.78 nM. (B) Visual color changes corresponding to (A). (C) TEM images of 2.78 nM AuNPs solution mixed with 83 nM RBA for 5 min after the addition of 0.138 M NaCl. (D) TEM images of 2.78 nM AuNPs solution in the presence of 83 nM RBA and 3.08 nM ricin for 5 min after the addition of 0.138 M NaCl.

### 3.3. Optimization of the key parameters

In this study, the absorption ratio between 670 nm and 520 nm, Δ(A670/A520)[Δ(A670/A520)=the absorption ratio A670/A520 in the presence of ricin - the absorption ratio A670/A520 in the absence of ricin], was used to optimize the key parameters. The Δ(A670/A520) value was mainly influenced by the concentration of RBA, the concentration of NaCl, and the reaction time after adding NaCl.

#### 3.3.1. Optimization of the amount of aptamer adsorbed on AuNPs

100 μL of 13 nm AuNPs was mixed with 15 μL of different concentration (0.5, 1, 2, 5 μM) RBA. Then, 15 μL of 2.3 nM ricin or PBS (0 nM ricin) was added to the AuNPs/RBA solution. The solutions were allowed to react for 5 min and then 50 μL of 0.5 M NaCl was added to produce color change. After the solution was equilibrated for 5 min, the resulting solution was transferred to a quartz cuvette. The Δ(A670/A520) reached the maximum value when the concentration of RBA was 1 μM. Hence, the concentration of RBA was selected to be 1μM for this experiment (ESI, Fig.S2†).As expected, the sensitivity was reduced with the amount of RBA increasing and this fit to the theory that ricin compete with AuNPs for binding to RBA and the least protected AuNPs amount of RBA means the most sensitivity. However, 0.05 μM RBA is too small to protect AuNPs from salt - induced aggravation even without ricin.

#### 3.3.2. Optimization of the concentration of salt

100 μL of 13 nm AuNPs was mixed with 15 μL of 1 μM RBA. Then, 15 μL of 2.3 nM ricin or PBS (0 nM ricin) was added to the AuNPs/RBA solution. The solutions were allowed to react for 5 min and then 50 μL of different concentration NaCl (0.1, 0.2, 0.5, 0.75, 1 M) was added to produce color change. After the solution was equilibrated for 5 min, the resulting solution was transferred to a quartz cuvette. The results showed that Δ(A670/A520) arrived at the maximum value when the concentration of NaCl was 0.5 M. Thus, 0.5 M was chosen for this study (ESI, Fig.S3†). 0.1 M and 0.2 M concentration of NaCl could not induce effective aggregation of AuNPs in the presence of ricin. 0.75 M and 1 M concentration of NaCl could induce aggregation of the AuNPs even without the presence of ricin.

#### 3.3.3. Optimization of the reaction time after adding NaCl

100 μL of 13 nm AuNPs was mixed with 15 μL of 1 μM RBA. Then, 15 μL of 2.3 nM ricin or PBS (0 nM ricin) was added to the AuNPs/RBA solution. The solutions were allowed to react for 5 min and then 50 μL of 0.5 M NaCl was added to produce color change. The reaction time after adding NaCl over the range of 0–30 min was studied. It was shown that the Δ(A670/A520) increased substantially as the reaction time increased up to 5 min. Therefore, 5 min was chosen as the reaction time (ESI, Fig.S4†).

### 3.4. Colorimetric biosensing of ricin

To detect ricin using colorimetric biosensor, a series of different concentrations of ricin was respectively added and their UV–vis spectra were recorded (Fig. 3A). Fig. 3B depicts the derived calibration curves corresponding to Fig. 3A. As can be seen in Fig. 3A and B, the absorption ratio, A670/A520, increased proportionally with the concentration of ricin in the range of 0.31–11.55 nM. The linear equation could be fitted as *A670/A520*=0.515 + 0.657 logC (*ricin, nM*) ($R^2$= 0.993). The detection limit can reach as low as 0.31 nM, which is lower than many previous reports. In addition, we compared the detection limit and detection time of the reported method. As shown in Table1. The detection sensitivity of the proposed method is higher or comparable than the previous reports. Moreover,the detection time is much shorter than many previous reports. Most of the reported methods need tedious pretreatment while our method does not need to prepare.Therefore,our method is simple and fast.

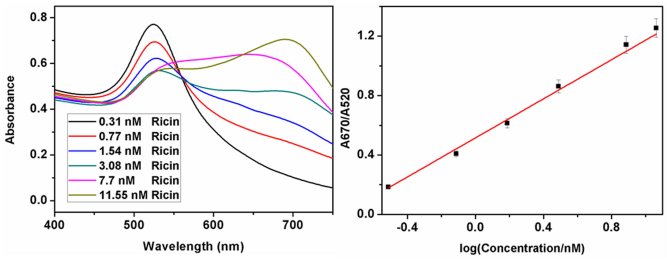


**Fig.3** (A) Absorption spectra of AuNPs in the presence of various concentration of ricin. (B) Typical calibration curve for ricin obtained using the aptamer-based biosensor.

**Table 1** Comparison of different method for ricin detection.

| Detection method | Detection Limit | Detection time (including Pre-treatment time) |
|---|---|---|
| Fluoroimmunoassay [32] | 1000 ng/ml | 12 h |
| Aptamer arrays biosensor assay [33] | 320 ng/ml | 6 h |
| SPR biosensor assay [34] | 200 ng/ml | 20 min |
| Immunochromatographic assay [12] | 50 ng/ml | 3 h |
| Aptamer biosensor assay [35] | 25 ng/ml | 21 h |
| Microarray biosensor assay [36] | 10 ng/ml | 4 h |
| Colorimetric biosensor assay [13] | 4 ng/ml | 2 days |
| ELISA [37] | 400 pg/ml | 20 h |
| Electrochemiluminescent assay [37] | 50 pg/ml | 11 h |
| Nanoparticle-based bio-barcode assay [38] | 1 fg/ml | 40 h |
| Nanoelectrode array biosensor assay [39] | Not given | 3.5 h |
| Aptamer-based colorimetric biosensor assay （This work） | 20 ng/ml | 1 h |

### 3.5. Selectivity

The selectivity of this biosensor to ricin was evaluated by measuring the absorption ratio value, A670/A520 to some different proteins such as thrombin (Th), horseradish peroxidase (HRP), lysozyme (Lys), glucose oxidase ($GO_X$), and bovine albumin (BSA). As can be observed in Fig. 4A, upon the addition of ricin, there was an obvious change in UV–vis adsorption spectrum, while no or just a little spectral change occurred in the absence (blank) or presence of the different proteins. The data derived from Fig. 4A showed that the adsorption ratio value, A670/A520, in the presence of ricin was considerably larger than those of blank or other proteins (Fig. 4B). All results indicated that our assay approach had a high specificity to ricin.

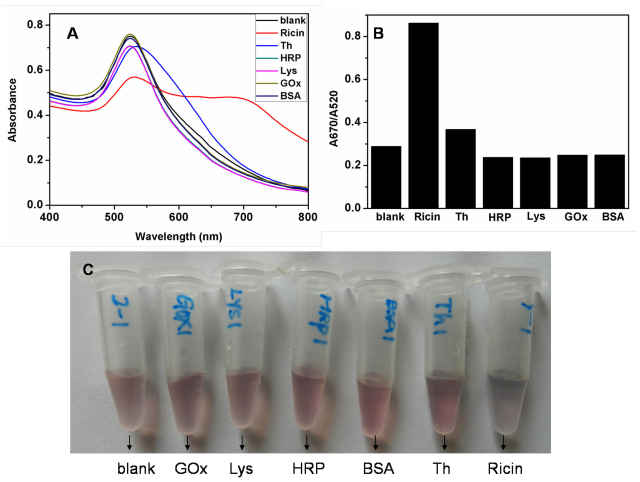


**Fig.4** (A) UV-vis absorption spectra of AuNPs in the presence of 83 nM RBA and 3.1 nM ricin or other different proteins after the addition of 0.138 M NaCl, $C_{AuNPs}$ = 2. 8 nM. (B) The absorption ratio value A670/A520 of ricin and other different proteins. Experimental conditions are the same as in (A). (C) Visual color changes corresponding to (A).

### 3.6. Application in real samples

In order to evaluate the feasibility of the present method in practical applications, the detection of ricin in milk powder and Pepsi Cola was carried out. The real samples were spiked with certain amounts of ricin. Table 1 shows that the recoveries of the real samples are in the range 104.6% to 127.8%. The desirable recoveries definitely demonstrate the reliability of the proposed method for detection of ricin in practical applications.

**Table 2** Analytical results for ricin in Pepsi Cola and Milk powder samples

| Sample | Add (nM) | Found (nM) a | Recovery (%) | RSD (%) |
|---|---|---|---|---|
| Pepsi Cola | 7.70 | 9.84±0.08 | 127.8 | 0.82 |
| | 11.55 | 12.21±0.03 | 105.7 | 0.25 |
| Milk powder | 7.70 | 9.50±0.11 | 123.4 | 1.20 |
| | 11.55 | 12.08±0.12 | 104.6 | 1.02 |

[a]Average of three determinations± standard deviation

## Conclusions

In summary, we have successfully developed a sensitive, accurate and reliable method for the detection of ricin by using aptamers and unmodified AuNPs. The red-to-blue color change of AuNPs in the presence of ricin was found to be easily observed by the naked eye or measured by UV–vis spectrometer. The linear dynamic range and its detection limit were found to be 0.31 nM to 11.55 nM and 0.31 nM, respectively. More importantly, the proposed method is successfully applied to the detection of ricin in real samples. Therefore, this method may offer a new approach for developing simple, low cost and sensitive sensors for ricin detection.

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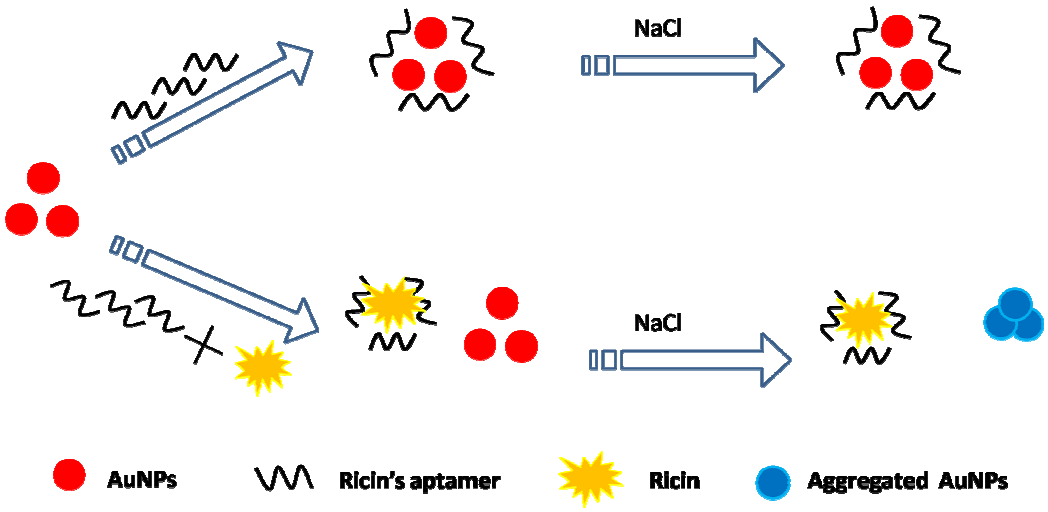


A sensitive aptamer-based colorimetric biosensor for the detection of ricin using unmodified gold nanoparticles as probe was developed .