Supporting information

Isolation high affinity ssDNA aptamer for the detection of ribavirin in chicken

Mingyan Song[ab], Chen Lyu[ab], Nuo Duan[abc], Shijia Wu[abc], Imran Mahmood Khan[abc], Zhouping Wang[abcd*],*

[a]State Key Laboratory of Food Science and Technology, Jiangnan University, Wuxi ,214122,China

[b]School of Food Science and Technology, Jiangnan University, Wuxi 214122, China,

[c]International Joint Laboratory on Food Safety, Jiangnan University, Wuxi 214122,

China

[d]National Engineering Research Center for Functional Food, Jiangnan University, Wuxi, Jiangsu 214122, China

* Corresponding author: Nuo Duan, Zhouping Wang

Tel. / Fax: +86-510-85917023

E-mail address: duannuo@jiangnan.edu.cn; wangzp@jiangnan.edu.cn

Postal address: No 1800 Lihu Avenue, Wuxi, Jiangsu, 214122, P. R.C.

ORCID

Nuo Duan: 0000-0001-6493-4951

Zhouping Wang: 0000-0002-3868-8125

Synthesis of Fe_3O_4 magnetic beads and avidin coated Fe_3O_4 magnetic beads

The Fe_3O_4 magnetic beads were prepared by a one-step solvothermal method according to report. Typically, 6.5 g of 1,6-hexanediamine, 2.0 g of anhydrous sodium acetate and 1.0 g of $FeCl_3 \cdot 6H_2O$ were dissolved in 30 mL of glycol to form a transparent solution with stirring vigorously at 50 °C. The mixture was subsequently transferred into a Teflonlined autoclave and heated to 198 °C for 6 h. The product was cooled to room temperature and then washed with water and ethanol followed by drying at 50 °C.

Then the magnetic beads coated with the avidin based on classical glutaraldehyde method. Briefly, 1 mg of the beads was dispersed in 1 mL of 10 mM phosphate buffer solution (PBS, pH 7.4), and 0.25 mL of 25% glutaraldehyde was added into the solution. The reaction was continued for 2 h at room temperature gentle shaking, followed by washing three times with washing buffer. The resultant beads were dispersed in 1 mL of 10 mM PBS, and 100 μL of avidin (1 mg mL^{-1}) were added and incubated for 2 h at 37 °C with gentle shaking. Next, the avidin coated Fe_3O_4 magnetic beads were magnetically collected and rinsed more times with PBS.

Synthesis of AuNPs

Add 1 mL of 1% chloroauric acid aqueous solution to a three-necked round bottom flask containing 98 mL of ultrapure water, stir vigorously and boil it in an oil bath, quickly add 1 mL of 5% trisodium citrate aqueous solution, after 30 minutes of reaction, the color of the solution Gradually change from light yellow to dark wine red, transfer the round-bottom flask to room temperature, stir and cool to room temperature, and store the prepared AuNPs solution at 4˚C away from light for later use.

Optimization of $MgCl_2$ concentration

When the salt concentration is too high, the gold nanoparticles may directly aggregate without a target. If the salt concentration is too small, the gold nanoparticles may partially aggregate and affect the experimental results. Therefore, the salt ($MgCl_2$) concentration needs to be optimized. It could be seen from the Figure S2 that when the concentration of $MgCl_2$ was 50mmol, the absorbance at 520nm decreased most obviously, and gold nanometers happened to aggregate, so 50mmol/L was chosen as the optimal concentration.

Synthesis of Carbon dots

Candle soot (8 mg) was suspended in 20 mL of mixed solvent (Vwater/Vethanol = 1:1), and the solution was sonicated for 4 hours. Then, the black mixture was centrifuged with 3000 rpm for 2 min to remove large-size particles. The supernatant was collected and centrifuged again for 6 min with 6000 rpm. A black precipitate, with a dry weight of ca. 2 mg, was obtained and dissolved in 20 mL of water containing and the concentration of CNPs was calculated as ca. 0.1 mg/mL.

Optimization of carbon dots concentration

To ensure that the fluorescence of unbound aptamers can be completely quenched by carbon dots, the concentration of carbon dots is optimized. Different amounts of carbon dots were added to 100nmol aptamer solution, and the final concentrations were respectively 0.005,0.01,0.02,0.03,0.04,0.05mg/mL. The blank group used buffer instead of carbon dots. It could be seen from the figure that the F value increased with the continuous increase of the concentration of carbon dots. When the concentration reached 0.04mg/mL, the quenching was complete, so 0.04mg/ml was chosen as the optimal concentration.

Figure options

Fig. S1 The illustration of the binding assay based on AuNPs

Fig. S2 Optimization of $MgCl_2$ concentration

Fig. S3 Optimization of carbon dots concentration

Fig. S4 Secondary structure of aptamer APT-2, APT-3, APT-4, APT-5, APT-6 and APT-7

Fig. S1

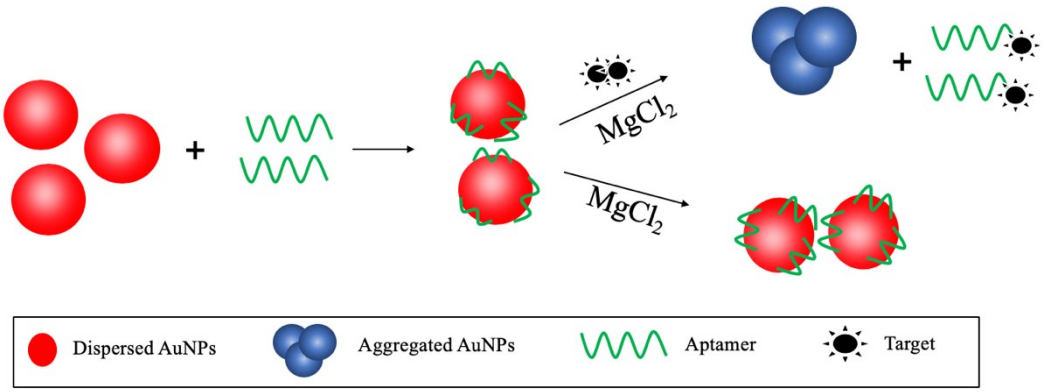

Fig. S2

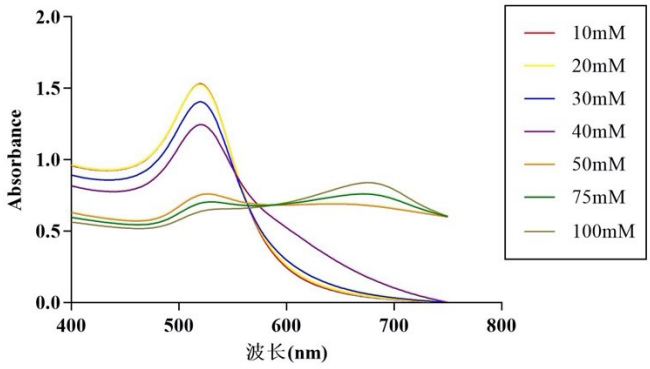

Fig. S3

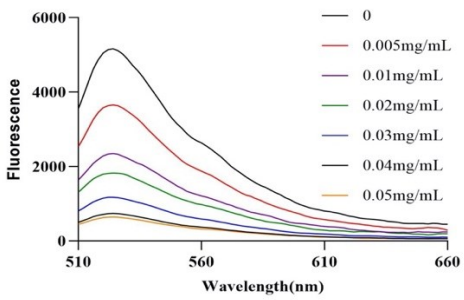

Fig. S4

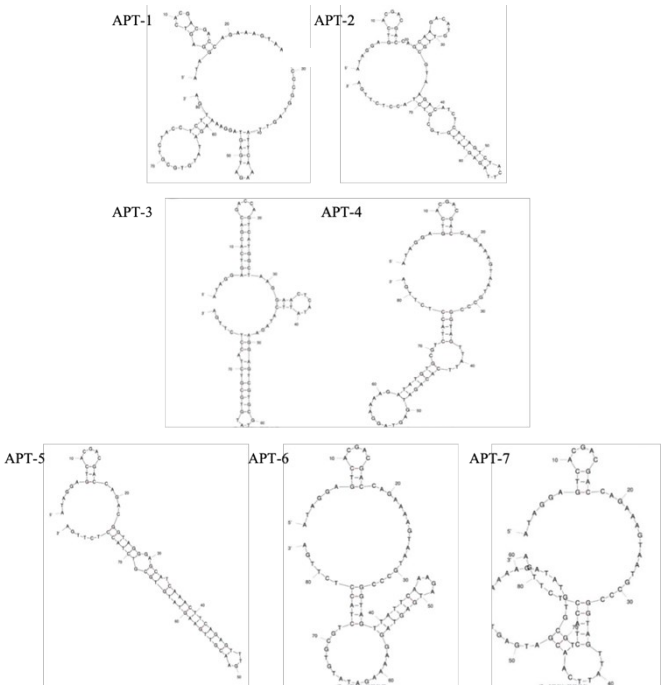

Table S1 The conditions for aptamers selection

Selection round	ssDNA (pmol)	Incubation time(min)	Concentration of ribavirin(pmol/μL)	Concentration of counter targets(pmol/μL)
1	1000	120	200	No
2	100	120	200	No
3	100	120	200	No
4	100	120	200	No
5	100	120	200	No
6	100	120	200	80
7	80	120	160	No
8	80	120	160	60
9	80	90	160	No
10	60	90	120	No
11	60	90	120	60
12	60	90	120	No
13	40	60	80	40
14	40	60	80	No
15	20	30	40	20