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Supplementary material

Immunocolorimetric assay based on amplified gold nanoparticles and magnetic separation beads for detection of sesame allergens in food

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1. Preparation of growth solution

1.1 Optimization of the CTAB concentration in seed growth solution

First, 22.8–409.0 mg of CTAB was dissolved into 5 mL of water. Separately, 12.5 μL of 0.1 M $HAuCl_4$ was added to CTAB solution at 37℃. After the addition of 65 μL of 100 mM AA, 6 μL AuNPs-Ab was added to 100 μL of seed solution for 10 min. Finally, 20 μL of sodium thiosulfate was added to stop the reaction.

1.2 Optimization of the $HAuCl_4$ concentration in seed growth solution

First, 2 mL of 100 mM CTAB was added into 0.5–9 μL of 0.1 M $HAuCl_4$ at 37℃, followed by the addition of 30 μL of 100 mM AA solution to make the final concentration of $HAuCl_4$ in seed growth solution between 0.025–0.45 mM. Subsequently, 6 μL AuNPs-Ab was added to 100 μL seed solution. After 10 min, 20 μL of sodium thiosulfate was added to stop the reaction.

1.3 Optimization of the AA concentration in seed growth solution

First, 2.5 μL of 0.1 M $HAuCl_4$ was added to 1 mL of 100 mM CTAB at 37°C, and 10–26 μL of 100 mM AA was added into the solution. Thus, the concentration of AA in seed growth solution was in the range of 1.0–2.6 mM. Then, 6 μL AuNPs-Ab was then added to 100 μL of seed solution. After 10 min, 20 μL of sodium thiosulfate was added to stop the reaction.

Table S1 Optimization of seed growth solution

Number	CTAB optimization		$HAuCl_4$ optimization		AA optimization	
	CTAB (mg)	CTAB (mM)	0.1 M $HAuCl_4$ (μL)	$HAuCl_4$ (mM)	100 mM AA (μL)	AA (mM)
1	22.8	12.5	0.5	0.025	10	1.0
2	45.6	25	1	0.05	11	1.1
3	91.0	50	2	0.1	12	1.2
4	136.7	75	3	0.15	13	1.3
5	182.2	100	4	0.2	14	1.4
6	227.8	125	5	0.25	15	1.5
7	273.3	150	6	0.3	16	1.6
8	318.9	175	7	0.35	17	1.7
9	364.5	200	8	0.4	18	1.8
10	409.0	225	9	0.45	20	2.0
11	–	–	–	–	22	2.2
12	–	–	–	–	24	2.4
13	–	–	–	–	26	2.6

– means no description

2. Mechanism of seed growth

In the presence of CTAB, Au^{3+} formed a stable $CTA^+[AuBr_4]^-$ complex with CTAB, as shown by the orange color of the solution (step 1 in Figure S1A and Figure S1B). After the addition of AA, the $CTA^+[AuBr_4]^-$ complex was quickly reduced to $CTA^+[AuBr_2]^-$, resulting in a colorless solution (step 2 in Figure S1A and Figure S1B). As shown in Figure S1C, $HAuCl_4$ had no absorption between 300 nm and 600 nm. In the absence of CTAB, Au^{3+} ions could be directly reduced to Au^0 by AA, and an absorption peak appeared at 553 nm, indicating the formation of Au^0. In the presence of CTAB, a significant absorption peak appeared at 394 nm, and the shoulder peak was located at approximately 462 nm. This may have been due to the ligand exchange between $HAuCl_4$ and CTAB. Finally, the AuNPs-Ab conjugate acted as a self-catalyst, accepting electrons and transferring them to Au^+ (step 3 in Figure S1A). Au^+ ions were reduced to Au^0, which was deposited on the surface of AuNPs-Ab, resulting in an increase in the size of the AuNPs. Finally, the addition of $Na_2S_2O_3$ stopped the growth of AuNPs by forming a stable complex between Au^+ and $Na_2S_2O_3$.

3. Optimization of seed growth solution

3.1 Optimization of CTAB concentration in seed growth solution

CTAB can stabilize reduced Au^+ and prevent the aggregation of amplified AuNPs. Therefore, the concentration of CTAB is critical for assay optimization. As shown in Figure S1D, when the CTAB concentration was 12.5 mM, the absorption peak was significantly red-shifted, by 16 nm. The absorption spectrum of amplified AuNPs-Ab (25 mM–75 mM) also had a slight red shift (2–3 nm). This might be because at low concentrations of CTAB, higher surface energy causes the aggregation of AuNPs-Ab. At a CTAB concentration of 100 mM, the UV absorption value of the amplified AuNPs-Ab was the highest and the solution was red (Figure S2A), indicating that no aggregation of AuNPs-Ab occurred. When the CTAB

concentration increased from 125 mM to 225 mM, the UV absorbance of AuNPs-Ab decreased and the absorbance intensity consequently decreased. Therefore, 100 mM CTAB was chosen for further experiments.

3.2 Optimization of the $HAuCl_4$ concentration in seed growth solution

The effect of $HAuCl_4$ concentration on the amplification of AuNPs-Ab was studied; the results are shown in Figure S1E. As the concentration of $HAuCl_4$ increased from 0.025 mM to 0.45 mM, the UV absorbance of AuNPs-Ab increased rapidly. When the $HAuCl_4$ concentration reached 0.25 mM, the UV absorbance of AuNPs-Ab was approximately 1.0, which was suitable for use in further experiments. However, although the absorbance increased from 0.25 mM to 0.45 mM, a slight redshift occurred. This may have been due to the large size of the amplified AuNPs-Ab when the $HAuCl_4$ concentration was high (the OD value was 1.2–3.1), and this high absorbance (OD > 1.2) resulted in inaccurate experimental results. The color changes in the experiment with different concentrations of $HAuCl_4$ are shown in Figure S2B. The addition of 5 μL of $HAuCl_4$ (0.25 mM) in the solution resulted in a burgundy appearance. The addition of 6–9 μL of $HAuCl_4$ (0.3–0.45 mM) was dark red, and the AuNPs may be slightly coagulated, which will affect the experimental results. Therefore, 0.25 mM $HAuCl_4$ was used for further experiments.

3.3 Optimization of the AA concentration in seed growth solution

During the AuNP amplification, $CTA^+[AuBr_4]^-$ was reduced to $CTA^+[AuBr_2]^-$ with AA, and $CTA^+[AuBr_2]^-$ was further reduced to Au^0 in the presence of AuNPs-Ab. As shown in Figure S1F and Figure S2C, as the concentration of AA increased from 1.0 mM to 1.5 mM, the UV absorbance increased rapidly. However, the absorption spectrum in the range of 1.6–2.6 mM had a slight redshift and the absorbance was unchanged. The color change in the experimental results with different concentrations of AA is shown in Figure S2C; 1.5 mM AA was selected for further study.

3.4 Optimization of incubation time of seed growth solution in AuNPs-Ab amplification

The incubation time for the seed growth solution to amplify AuNPs-Ab was optimized (Figure S1G). As the reaction time increased from 0 min to 10 min, the UV absorbance of AuNPs-Ab gradually increased, and the maximum UV absorbance displayed a gradual redshift. The reason may be that the increase in the AuNPs size caused a change in the refractive index and aggregation. When the reaction time was increased from 11 min to 13 min, the absorbance of the amplified AuNPs-Ab did not change; therefore, the optimal reaction time was set as 10 min. The absorbance of AuNPs-Ab remained unchanged after the addition of 20 μL of 10 mM $Na_2S_2O_3$.

3.5 Stability of seed growth solution

To eliminate the effect of different batches of seed growth liquid on gold nanoparticles, we evaluated the stable growth of gold nanoparticles from the seed growth solutions (n=6). The results showed that the signal amplified by AuNPs-Ab was stable each time (Figure S1H). For the six batches, the absorbance of AuNPs-Ab with and without seed growth solution was constant.

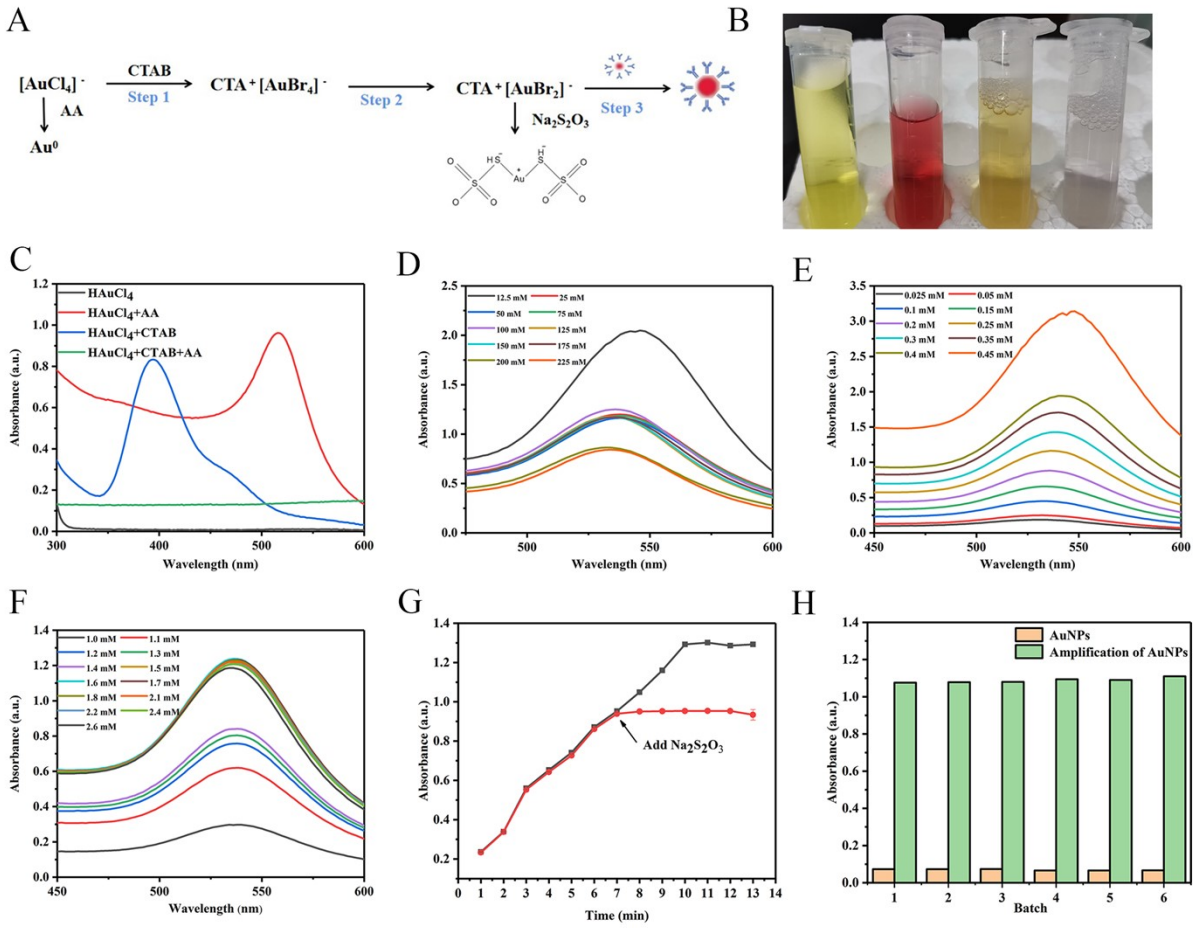

Figure S1. Optimization of working parameters of seed growth liquid. Schematic diagram of seed growth solution (A); Color diagram of $HAuCl_4$, $HAuCl_4$+AA, $HAuCl_4$+CTAB, $HAuCl_4$+CTAB+AA (B); UV absorption spectrum of $CHAuCl_4$, $HAuCl_4$+AA, $HAuCl_4$+CTAB, $HAuCl_4$+CTAB+AA (C); The absorbance values of seed growth solution with different concentrations of CTAB (D); The absorbance values of seed growth solution with different concentrations of $HAuCl_4$ (E); The absorbance values of seed growth solution with different concentrations of AA (F); The absorbance values of AuNPs and seed growth solution over 13 min (with $Na_2S_2O_3$ (red dot) and without $Na_2S_2O_3$(black dot)) (G); The absorbance value of six batches of seed growth solution (H).

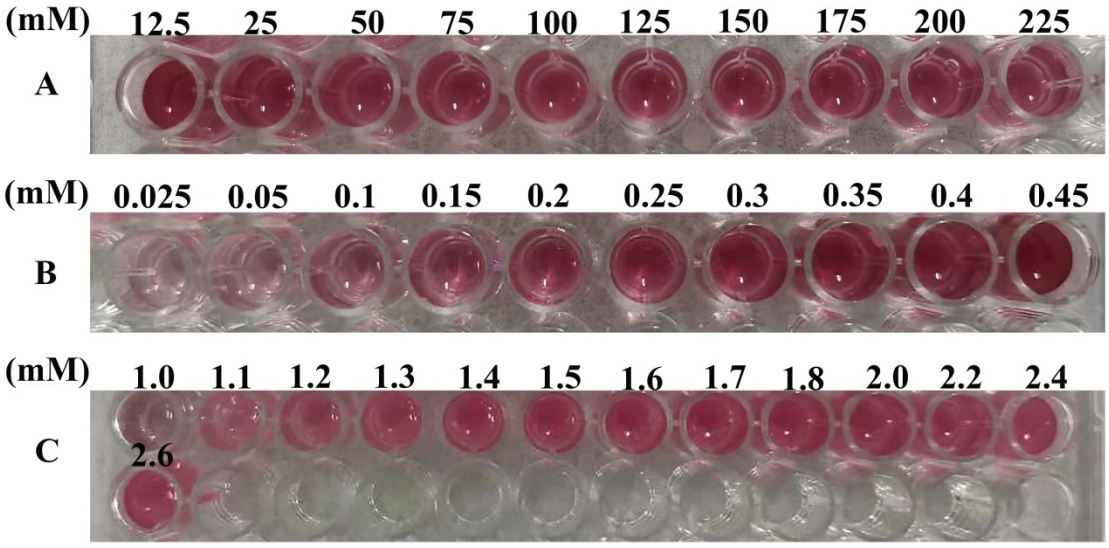

Figure S2 Optimization of the conditions of seed growth solution. The color of the seed growth solution with different concentrations of CTAB (A). The color of the seed growth solution with different concentrations of $HAuCl_4$ (B). The color of the seed growth solution with different concentrations of AA (C).

4. Optimization of the amount of sesame modified on MPMs

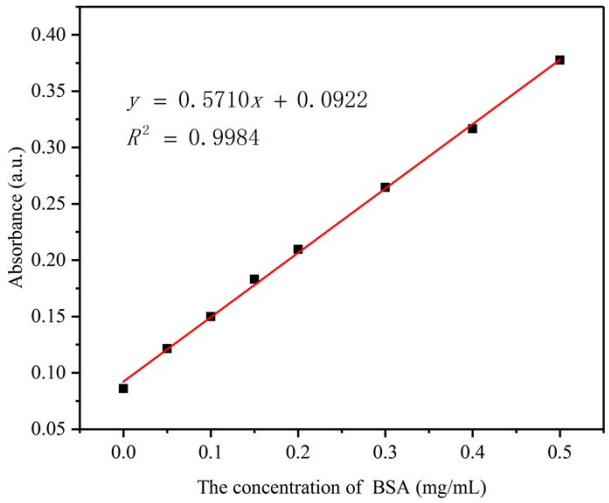

Figure S3 The standard curve of BCA kit.