A highly sensitive resonance Rayleigh scattering method for hemin based on the aptamer-nanogold probe catalysis of citrate-$HAuCl_4$ particle reaction

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Experimental

Apparatus and Reagents

A F-7000 fluorescence spectrophotometer (Hitachi Company, Japan), a 79-1 magnetic stirrer with heating (Zhongda Instrumental Plant, Jiangsu, China), a JSM-6380LV scan electron microscope (Electronic Co. Ltd, Japan), and a TU-1901 type double beam UV-visible spectrophotometer (Beijing Purkinjie General Instrument Co., Ltd) were used.

A 1μmol/L hemin aptamer (ssDNA1) (Biological Technology Co., Ltd., Shanghai, China) with a sequence of 5-GTGGGTAGGGCGGGTTGG-3, ssDNA2 of 5′-TGGGGGTTGAGGCTA-3′, ssDNA3 of 5′-TGGGGGTTGAGGCTAAGCCGAAGCCGA-3′ were used. A 1% $HAuCl_4{\cdot}4H_2O$ $HAuCl_4{\cdot}4H_2O$ (Sinopharm Chemical Reagent Co., Ltd), a 1mol/L HCl solution, pH 8.0Tris-HCl buffer solution (50mmol/L), 2 mol/L NaCl and 1% trisodium citrate solution were prepared. A 1.5 mmol/L hemin stock solution: 0.1000g hemin (Sigma chemical company, America) was dissolved in 10 mL pH 8.0 Tris solution (0.2mol/L) and diluted with water (100mL). The working solutions of 10μmol/L hemin were freshly prepared by dilution of the hemin stock solution with water. All regents were of analytical grade and the water was doubly distilled.

AuNPs Preparation

In a 250mL round-bottom flask containing 50 mL water on the heat magnetic stirrer (2mot/min), 4.0mL of 1% trisodium citrate was added after the water boiling. Then 500μL of 1% $HAuCl_4$ was addedquickly and maintaining the water boiling. The solution turned red within 5min and the final color change to brilliant red. Continued Boiling for 10 min, the heating source was removed, and the colloid was not stirred until cold. Last the solution was transferred to a 50 mL volumetric flask and diluted to 50 mL with water (57.0μg/mL Au), then it was stored at 4℃.

Preparation of the AussDNA probe

A 2.0 mL of 1.0μmol/L ssDNA solution was added to a 8.0mL 58μg/mL AuNPs solution while stirring. The mixture was stirred for 10 min and stored at 4℃. The concentration, counted as ssDNA, was 0.20 μmol/L AussDNA.

Optimization conditions of the preparation of AussDNA probe

In a 5mL tube, 200μL 58μg/mL AuNPs solution, 200μLpH 8.0 Tris-HCl buffle solution and a certain concentration of NaCl solution were added solution successively, and diluted to 2.0mL, and mixed well. The mixture was placed in room temperature (25℃) for 10min, and the RRS intensity I_{368nm} was recorded. Results showed that when 50mmol/L NaCl was added, the value of I_{368nm} reached its maximum and keep stable following the NaCl concentration increased (Fig. 1S). So, a 50mmol/L NaCl was selected.

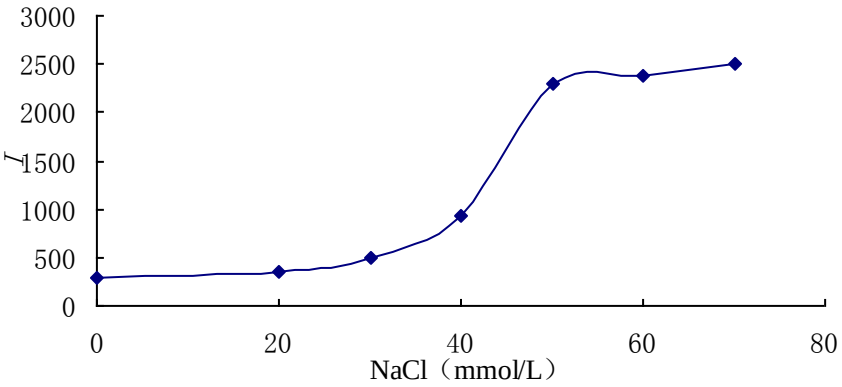


Fig. 1S Effect of NaCl concentration
5.8μg/mL AuNPs+2.5mmol/L Tris-HCl.

A certain of 1μmol/L ssDNA solution, 200μLpH 8.0 Tris-HCl buffle solution were added into a 5mL graduated, after keeping 10min later, added 50mmol/L NaCl solution, and diluted to 2.0mL, and mixed well. At room temperature (25℃), the RRS intensity I_{368nm} was recorded. Fig. 2S show that the RRS intensity value I_{368nm} reached its minimum and tends to be stable after added 25nmol/L ssDNA solution. Thus, a 25nmol/L ssDNA solution was chosen for use.

A 25nmol/L ssDNA solution and 200μL 58μg/mL AuNPs solution were added into a 5mL graduated tube, after keep for some time, then added 200μL pH 8.0 Tris-HCl buffle solution and 50mmol/L NaCl solution successively, and diluted to 2.0mL, and mixed well. According to the result, the interaction between ssDNA and AuNPs tends to be stable after 10min later. Thus, 10min at 25℃ was chosen as interact time.

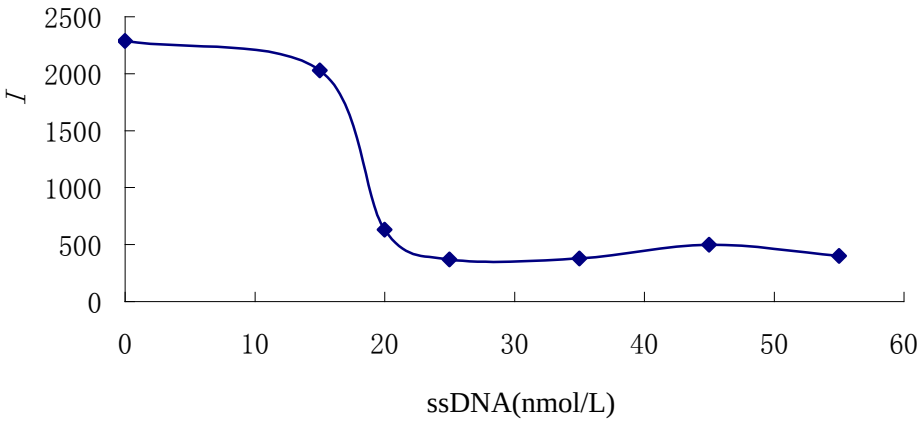
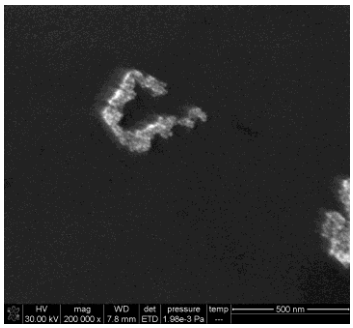
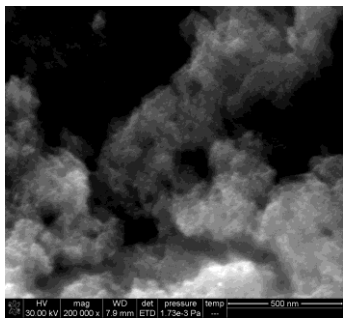


Fig. 2S Effect of ssDNA concentration
5.8μg/mL AuNPs +2.5mmol/L Tris-HCl +50mmol/L NaCl.



a



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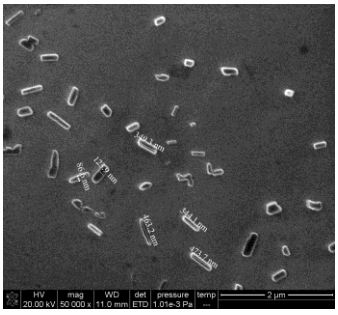

c

Fig. 3S Scanning electron microscope images

a: 25nmol/L AussDNA+1.25mmol/L Tris-HCl+50mmol/L NaCl; b: a+500nmol/L Hemin; c: 84μmol/L $HAuCl_4$+50mmol/L HCl+1.36mmol/L citrate+80μL aptamer reaction solution (100nmol/L Hemin).

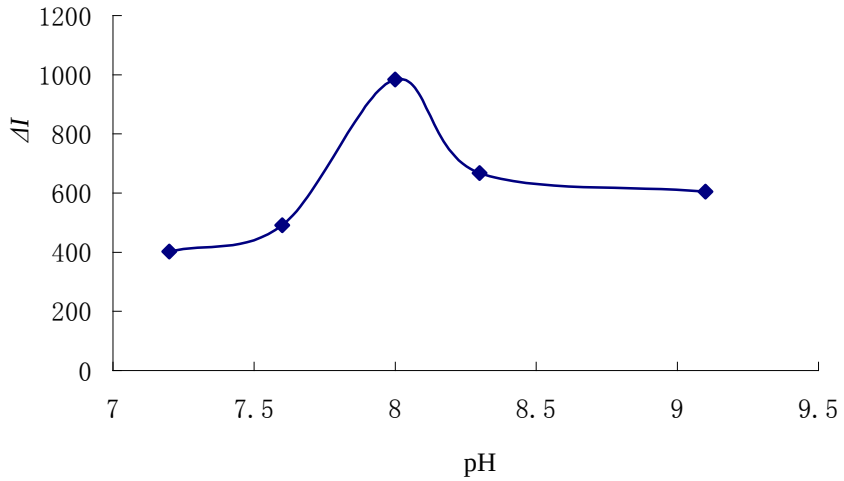

Fig. 4S Effect of Tris-HCl buffle solution pH

25nmol/L AussDNA1 +2.5mmol/L Tris-HCL +50mmol/L NaCl.

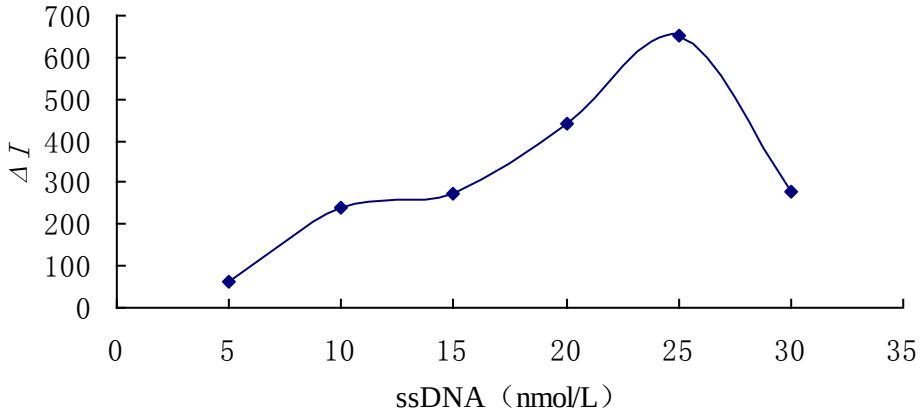

Fig. 5S Effect of AussDNA concentration

2.5mmol/L Tris-HCl+50mmol/L NaCl.

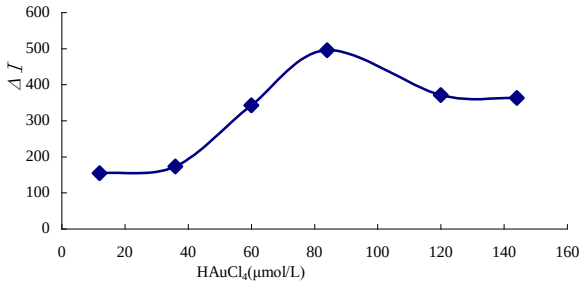

Fig. 6S Effect of $HAuCl_4$ concentration

50mmol/L HCl+120ng/mL AuNPs+1.36mmol/L citrate.

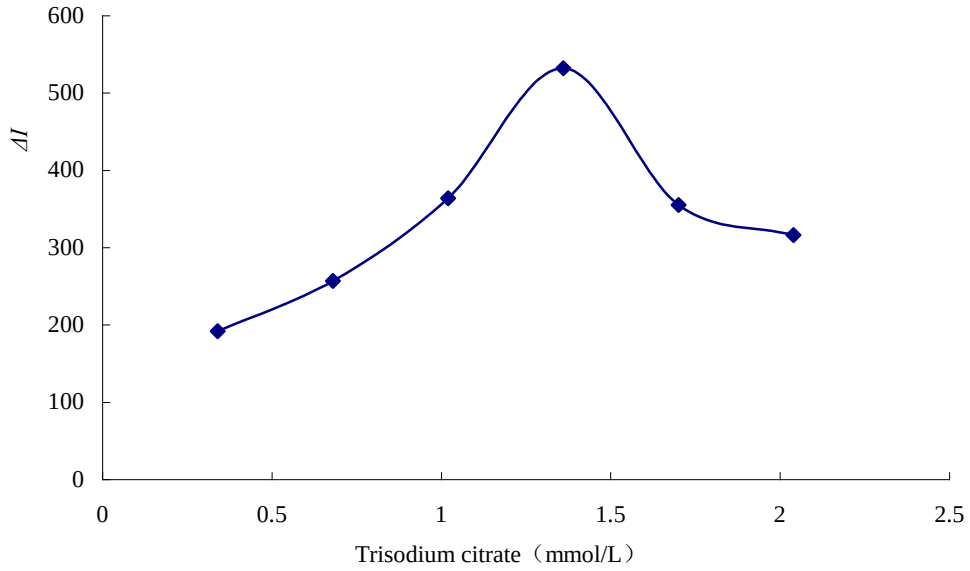

Fig. 7S Effect of trisodium citrate concentration

84μmol/L $HAuCl_4$+50mmol/L HCl+120ng/mLAuNPs+60℃+15min.

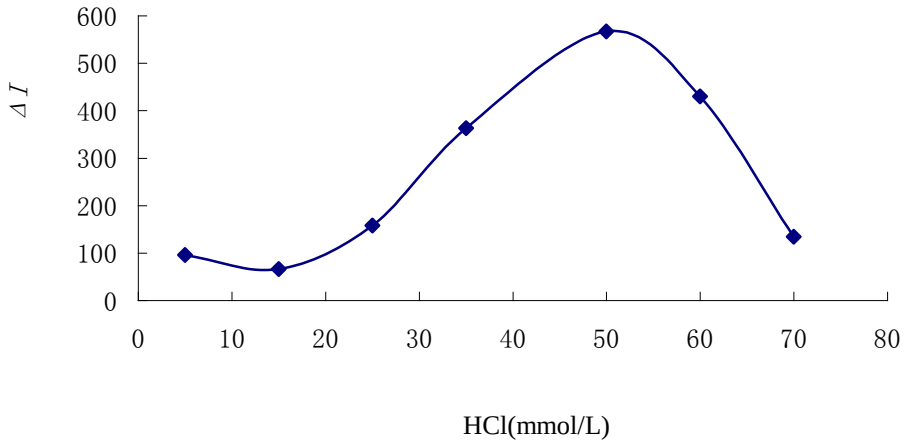

Fig. 8S Effect of HCl concentration

84μmol/L $HAuCl_4$+120ng/mL AuNPs+1.36mmol/L trisodium citrate.

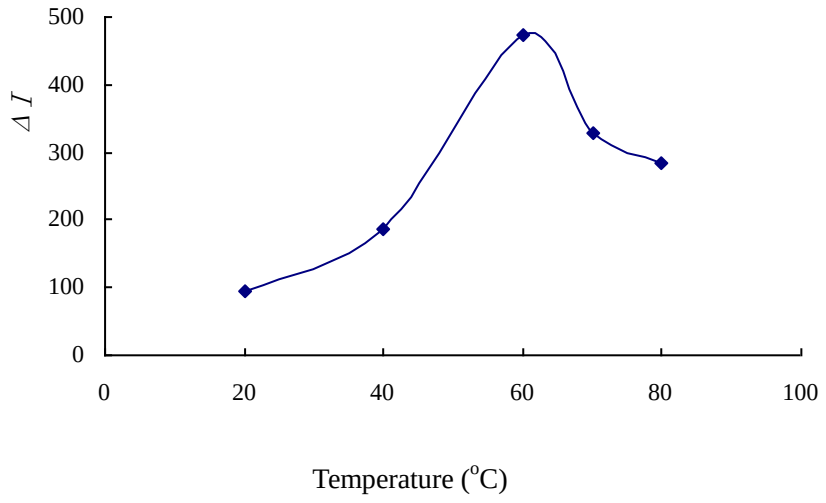

Fig. 9S Effect of reaction temperature

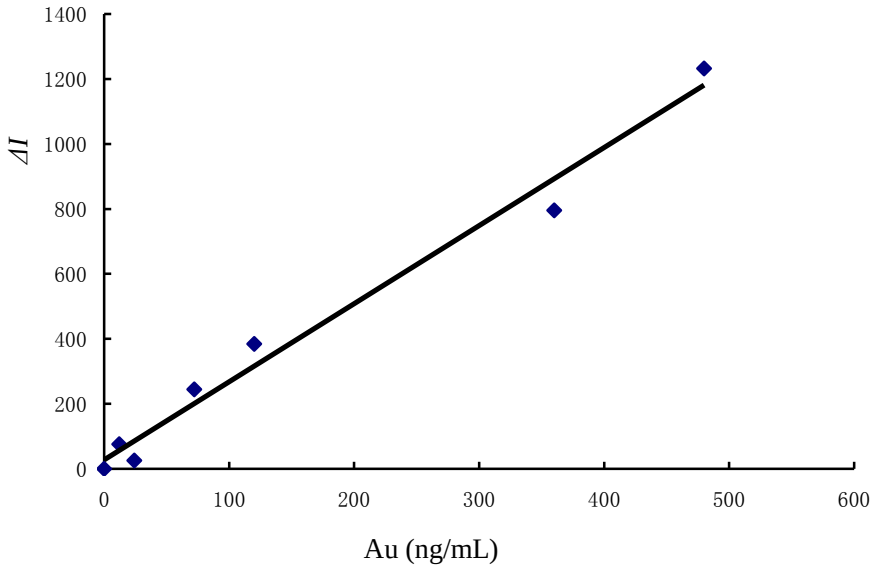

Fig. 10S The relationship between C_{AuNP} with $\Delta I_{370\ nm}$

84μmol/L $HAuCl_4$+50mmol/L HCl+120ng/mL AuNPs+1.36mmol/L trisodium citrate.

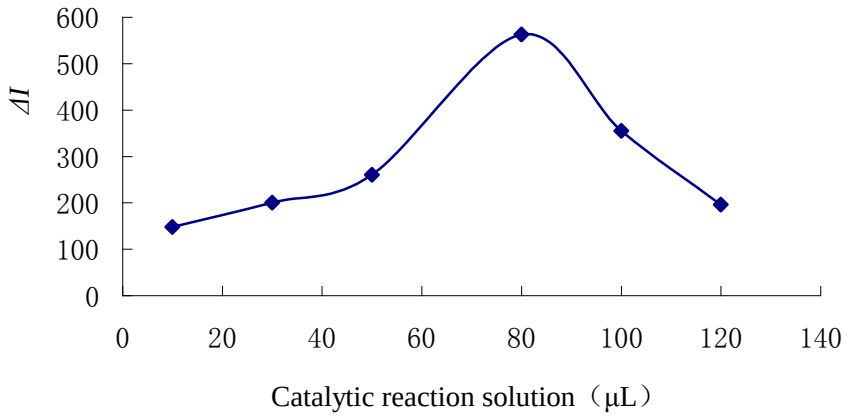

Fig. 11S Effect of catalytic reaction solution volume

84μmol/L $HAuCl_4$+50mmol/L HCl+1.36mmol/L trisodium citrate .

Table 1S Effect of coexistence ions

Coexistent substance	Tolerance (Times)	Relative error (%)	Coexistent substance	Tolerance (Times)	Relative error (%)
L-Lysine	200	-3	glucose	200	1
L-Cystine	150	6	V_{B1}	100	1
L- Phenylalanine	150	-2	K^+	200	-1
L- Tyrosine	100	5	Ca^{2+}	200	-6
L-Glutamic acid	100	2	Zn^{2+}	200	6
BSA	14	-2	Fe^{3+}	200	7
HSA	13	-1	Mg^{2+}	100	3

HAS: Human serum albumin; BSA: Bovine serum albumin.