Supporting Information for

Quantum dots-gold(III)-based indirect fluorescence immunoassay for high-throughput screening of APP

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Materials and Reagents:

Polystyrene 96-well microtiter plate was used to perform the immunoreaction. Rabbit anti-pig IgG was purchased from Biosynthesis Biotechnology Co., Ltd. (Beijing, China). Bovine serum albumin (BSA) was from German Roche Co. Ltd. (Germany). $HAuCl_4 \bullet 4H_2O$ was obtained from Shanghai Chemical Reagent Co. Ltd. (Shanghai, China). Peroxidase-conjugated rabbit anti-pig IgG conjugate and orthophenylinediamine dihydrochloride substrate were obtained from Sigma Chemical Co. (USA). The recombinant ApxIV protein, standard positive and negative sera were made by State Key Laboratory of Agricultural Microbiology (Huazhong Agricultural University). The positive control serum was a polyclonal anti-sera from a pig experimentally infected with APP. The negative control serum was obtained from an APP-free herd. Clinical sera were obtained from naturally infected and non-infected pigs. The buffers used were as follows: (A) coating buffer, 0.05 mol L^{-1} carbonate-bicarbonate buffer solution, pH 9.6; (B) incubation buffer, 0.1 mol L^{-1} sodium phosphate buffered saline (PBS), pH 7.2-7.4; (C) washing buffer, buffer B with 0.05% Tween 20; (D) dilution buffer, buffer B with 1% BSA. All other reagents were of analytical reagent grade and used as purchase. The fluorescence (FL) spectra were acquired with Perkin Elmer Model LS-55 luminescence spectrometer equipped with a 20 kW xenon discharge lamp as a light source. The FL signals were measured by a multifunctional ELISA reader with excitation wavelength at 390 nm and emission wavelength at 528 nm (Biotec). The ultraviolet and visible (UV-vis) absorption spectra were acquired on a Thermo Nicolet Corporation Model evolution 300 UV-vis spectrometer. The transmission electron microscopy (TEM) images of gold NPs and gold NPs-Rabbit anti-Pig IgG conjugate were acquired on an H-7650 TEM (Japan). The pH measurements were made with a Model pHS-3C meter (Shanghai Leici Equipment Factory, China).

Experimental process:

Recombinant ApxIV protein immobilization:

The recombinant ApxIV protein was diluted 650 times using buffer A and dispensed each 100 μL in appropriate wells of the 96-well polystyrene microplate. The wells were incubated overnight at 4 ℃. After washing with buffer C, a second coating was done by using 300 μL buffer D and incubated for 0.5 h at 37 ℃. The solution was discarded and wells were washed with buffer C, dried under vacuum and stored at 4 ℃.

Procedures for the immunoassay:

A series of dilutions of analyte (Pig sera) in buffer D (100 μL) were injected into wells and incubated for 1 h at 37 ℃. The wells were washed three times with buffer C, and 100 μL gold NPs-Rabbit anti-Pig IgG conjugate was added to each well. The wells were incubated with gentle shaking for 1 h at 37 ℃. Finally, the wells were washed with buffer C and super purified water three times.

Standard procedures for the FL quenching detection:

After the wells were thoroughly washed, gold NPs-Rabbit anti-Pig IgG conjugate was dissolved to form gold(III) with 100 μL per well of HCl-Br_2 solutions for 20 min to ensure that the gold NPs was dissolved completely. After oxidative treatment, the resultant mixture was placed in the oven drying by distillation to remove bromine and HCl, completely. Then 100 μL QDs was injected into the well. The FL signal was measured by the multifunctional ELISA reader.

ApxIV-ELISA:

The non-competitive indirect ELISA for the detection of antibody against ApxIV in sera of pigs was developed.[1] Briefly, the purified recombinant ApxIV protein was bound to 96-well

polystyrene microplate plates in a final concentration of 2.97 μg mL^{-1}. Serum samples were diluted 40 times using dilution buffer, the antibody in the serum samples was captured by the recombinant ApxIV protein and then sandwiched by peroxidase-conjugated Rabbit anti-Pig IgG conjugate. Next, the unbound peroxidase-conjugated Rabbit anti-Pig IgG conjugate was removed. 100 μL of freshly prepared 37 mg orthophenylinediamine dihydrochloride substrate in 10 mL of citric acid phosphate buffer (0.1 mol L^{-1}, pH 5.0), with 10 μL of 30% H_2O_2 were added to the wells. The plate was incubated at room temperature for 15 min. The reaction was then stopped with 50 μL of 2 mol L^{-1} sulphuric acid and the optical density was measured at 490 nm by ELISA reader.

Synthesis of Glutathione (GSH) Capped CdTe QDs:

In a typical synthesis, cadmium chloride ($CdCl_2$•$2.5H_2O$, 2.5×10^{-4} mol) was dissolved in 25 mL ultrapure water in a two-necked flask, and GSH (3.0×10^{-4} mol), trisodium citrate dihydrate (8.5×10^{-5} mol), Na_2TeO_3 (5.0×10^{-5} mol) and $NaBH_4$ (2.4×10^{-4} mol) were added at pH 11.0 with vigorous stirring. When the color of the solution changed to light green, the mixture was refluxed at 100 ℃ and the growth of CdTe QDs took place immediately.

Fig. S1 shows typical UV-vis absorption and FL spectra of the as-prepared GSH capped CdTe QDs. A particle size of 2.9 nm and concentration of 3.0×10^{-5} mol L^{-1} were estimated from UV-vis absorption spectrum, based on the first absorption peak wavelength.[2] Furthermore, the FL of GSH capped CdTe QDs exhibits a peak at 544 nm when excited at 390 nm.

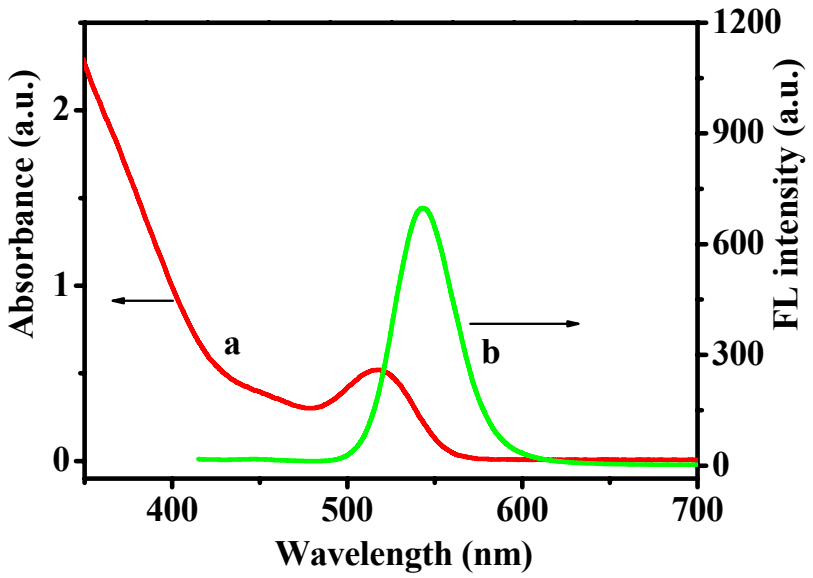

Fig. S1 The UV-vis (a) and FL (b) spectrum of the as-prepared GSH capped CdTe QDs. Excitation wavelength: 390 nm.

Synthesis of gold NPs:

Gold NPs were synthesized by the citrate reduction method.[3] All glassware used was thoroughly cleaned in HNO_3-HCl solution (3 parts HCl, 1 part HNO_3), rinsed with distilled water, and dried in an oven. Briefly, 0.01% solution (100 mL) of $HAuCl_4 \cdot 4H_2O$ was brought to boiling and 3 mL of 1% (wt) solution of sodium citrate was added to the boiling solution under stirring. After the color changed from light yellow to brilliant red, the solution was boiled for another 5 min to complete the reduction of the $HAuCl_4$. After cooling to room temperature, the gold NPs solution was diluted to 100 mL using deionized water. The resulting solution of gold NPs was characterized by UV-vis absorption spectrum (**Fig S2a**) and TEM (**Fig S3a**).

Preparation of gold NPs-Rabbit anti-Pig IgG conjugate:

Gold NPs-Rabbit anti-Pig IgG conjugate was prepared according to our previously reported method.[1] Gold NPs (50 mL) were mixed with the Rabbit anti-Pig IgG for 2 h at pH 9.0. The mixture solution was purified by centrifugation at 15000 g for 1 h, and the soft sediment was resuspended in sodium phosphatebuffered saline (0.1 mol L^{-1}, pH 7.2-7.4) and stored at 4 °C. The as-prepared gold NPs-Rabbit anti-Pig IgG conjugate was characterized by UV-vis absorption spectrum (**Fig S2b**) and TEM (**Fig S3b**).

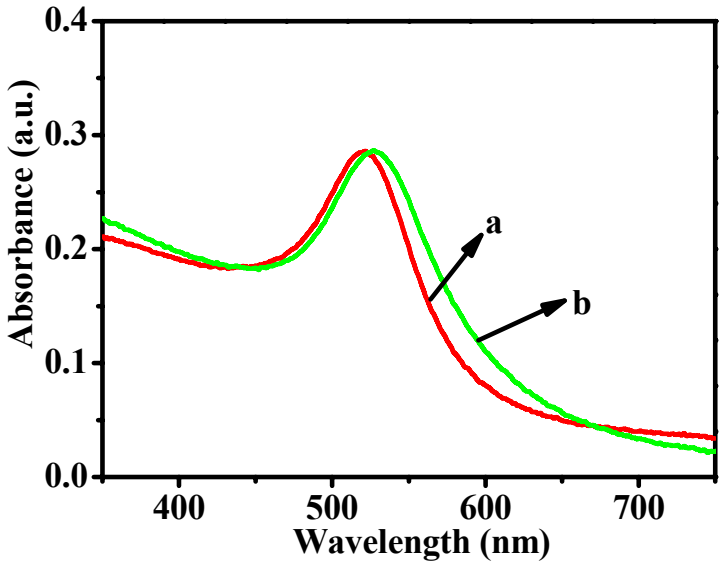

Fig S2 The UV-vis spectra of (a) gold NPs and (b) gold NPs-Rabbit anti-Pig IgG conjugate.

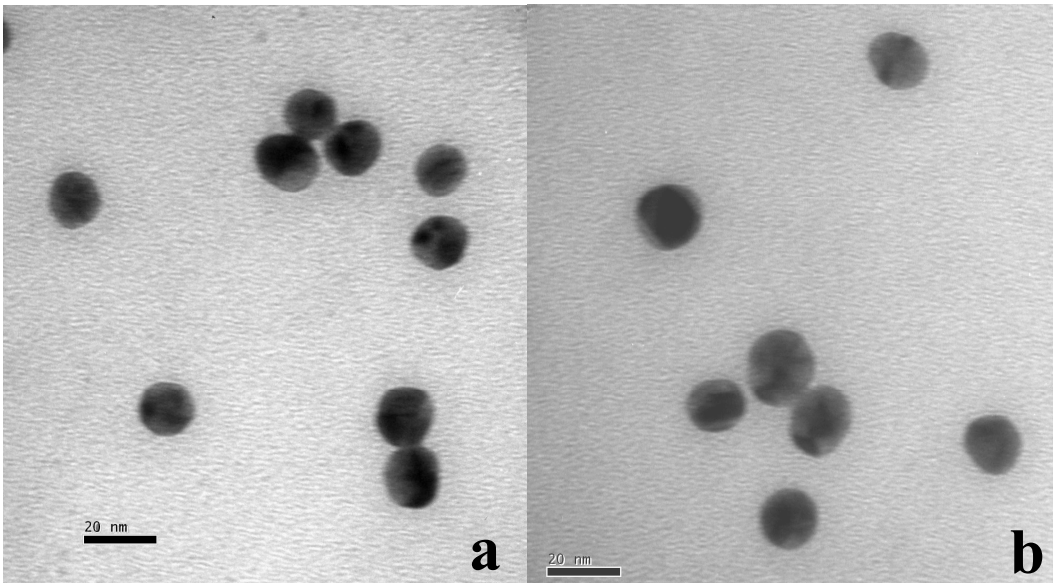

Fig S3 TEM images of (a) gold NPs and (b) gold NPs-Rabbit anti-Pig IgG conjugate.

Effect of gold NPs-Rabbit anti-Pig IgG conjugate and gold(III) on the luminescent intensity of GSH capped QDs:

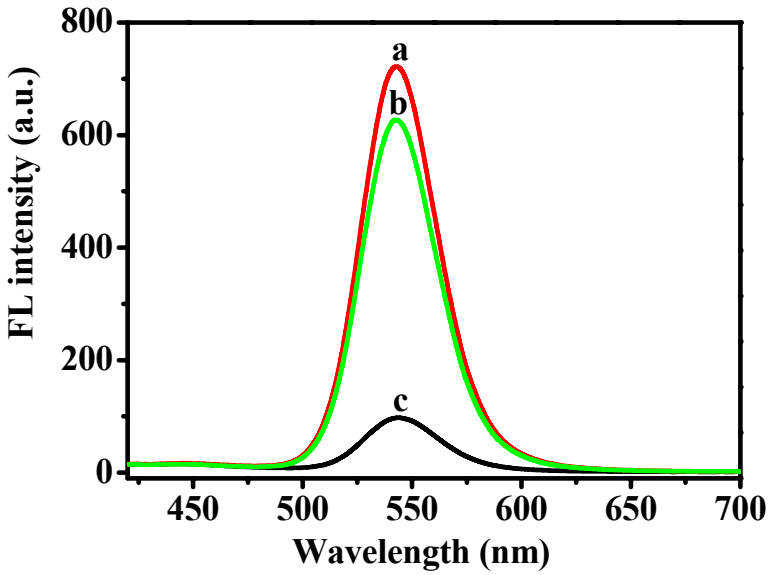

Fig S4 Effect of (b) gold NPs-Rabbit anti-Pig IgG conjugate and (c) gold(III), dissolved from gold NPs-Rabbit anti-Pig IgG conjugate, on the FL emission spectrum of (a) GSH capped CdTe QDs. The excitation wavelength is 390 nm (CdTe QDs: 3.0×10^{-7} mol L^{-1}; gold NPs-Rabbit anti-Pig IgG conjugate: 5.0×10^{-7} mol L^{-1}).

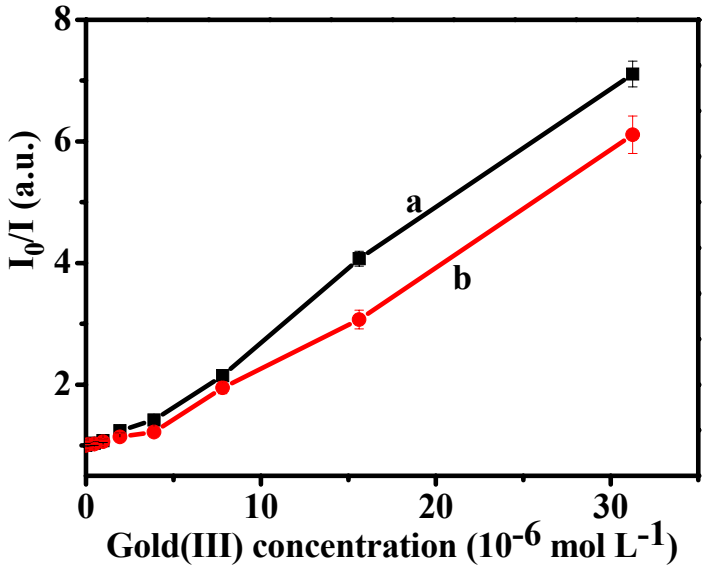

Fig S5 Effect of gold(III) concentration on the I_0/I of GSH capped CdTe QDs in the absence of free GSH (a) and presence of GSH (1.0×10^{-6} mol L^{-1}) (b).

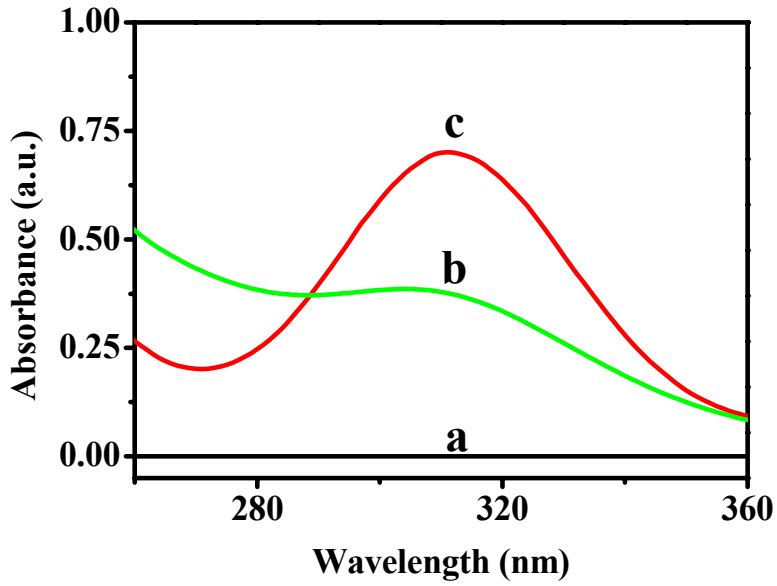

Fig S6 The UV–vis absorption spectra of (a) GSH, (b) GSH and gold(III) and (c) gold(III). (GSH: 2.0×10^{-5} mol L^{-1}, gold(III): 5.0×10^{-4} mol L^{-1}).

Optimizing dissolution conditions of gold NPs-Rabbit anti-Pig IgG conjugate:

The mixture solution of HCl and Br_2 was used to dissolve the gold NPs-Rabbit anti-Pig IgG conjugate to gold(III). The resultant mixture was placed in the oven at 60 ℃ to remove any remaining HCl and Br_2. Then, a 100 μL QD solution (7.5×10^{-8} mol L^{-1}) was added to a 96-well microplate and detected FL signals. The conditions of gold NPs-Rabbit anti-Pig IgG dissolution with the use of HCl-Br_2 have been studied. Firstly, as the concentration of HCl increased, the relative fluorescent intensity of CdTe QDs (I/I_0) decreased between 0.01 mol L^{-1} and 0.07 mol L^{-1}, and then maintained almost the same in the range of 0.07 to 0.2 mol L^{-1} HCl. Hence, subsequent work employed 0.1 mol L^{-1} HCl (Fig. S7). Secondly, the relative fluorescent intensity decreased and reached its balance value at 0.4 mol L^{-1}. Thus, 0.4 mol L^{-1} Br_2 was chosen for the following experiments (Fig.S8).

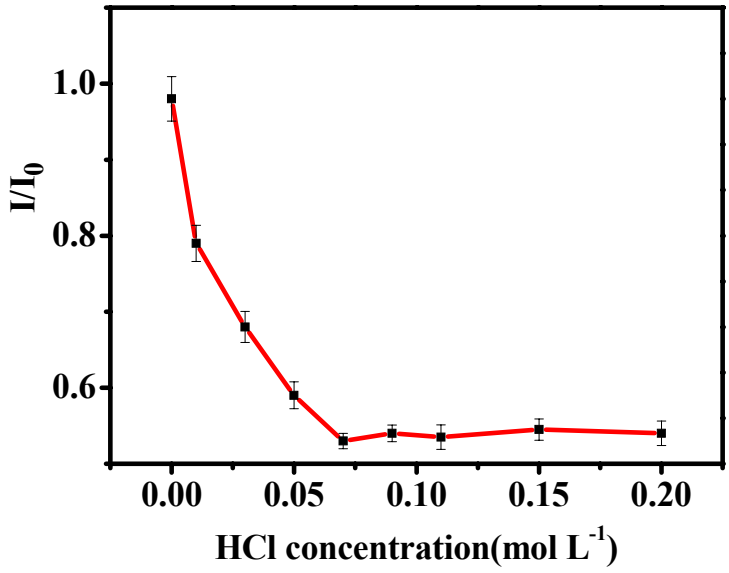

Fig.S7 Effect of dissolution conditions of HCl on the FL intensity of CdTe QDs (CdTe QD concentration: 7.5×10^{-8} mol L^{-1}, excitation wavelength: 390 nm)

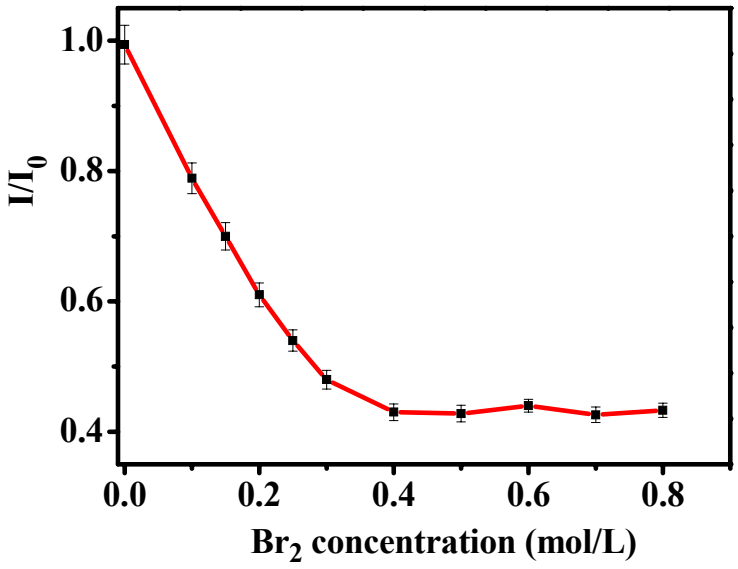

Fig.S8 Effect of dissolution conditions of Br_2 on the FL intensity of CdTe QDs (CdTe QD concentration: 7.5×10^{-8} mol L^{-1}, excitation wavelength: 390 nm)

The effect of reactive time on the interaction of CdTe QDs with gold(III):

The interaction of CdTe QDs and gold(III) was monitored at different time scales at room temperature. The relative FL intensity of CdTe QDs had a rapid decrease with increasing of the time until 40 min, which indicated that certain time is needed to complete the interaction. The reaction time of 40 min was chosen in the following experiments (Fig. S9):

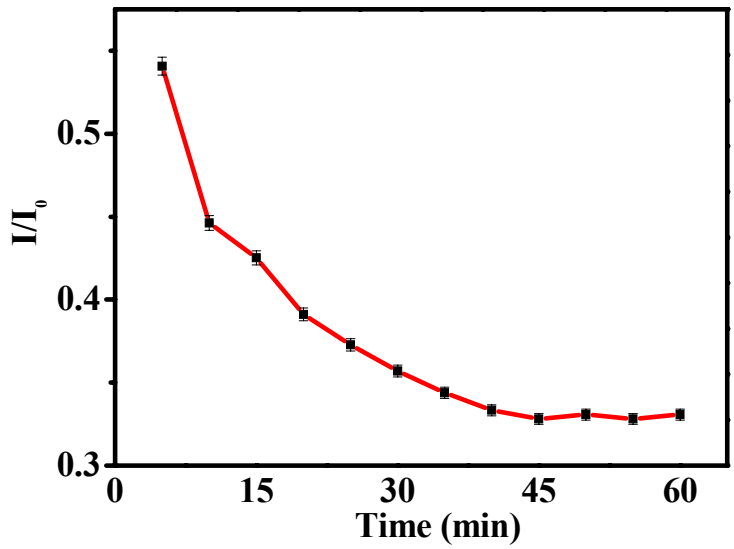

Fig.S9 Effect of reaction time on the reactive fluorescent intensity of CdTe QDs in the presence of gold(III) (CdTe QD concentration: 7.5×10^{-8} mol L^{-1}, gold(III): 5.0×10^{-6} mol L^{-1}, excitation wavelength: 390 nm).

ELISA method has been recognized as sensitive and specific methods used in diagnosis of APP. Therefore, in our experiment, ApxIV-ELISA was chosen as a reference test. First, it was used to successfully analyze 30 clinical serum samples. The results showed that 15 samples were positive and the other samples were negative. Then, the proposed method (indirect FLIA) was used to detect 15 positive and 15 negative clinical serum samples, respectively. The results were shown in **Table S1**.

Table S1 The results of the proposed method for the detection of 30 clinical serum samples

	The proposed method (Indirect FLIA)	
	Positive	Negative
15 positive serum samples	14	1
15 negative serum samples	2	13

The efficiency, sensitivity and specificity value were calculated using following equations:

Efficiency = (TP + TN) × 100/Total = 90.0 (TP = 14, TN = 13, Total = 30)

Sensitivity = TP × 100/(TP + FN) = 87.5; (TP = 14, FN = 2)

Specificity = TN × 100/(TN + FP) = 92.9; (TN = 13, FP = 1)

TP: True Positive; TN: True Negative; FP: False Positive; FN: False Negative.

Above calculation method has been used in our previous report.[1]

References:

1 D. H. Hu, H. Y. Han, R. Zhou, F. Dong, W. C. Bei, F. Jia, H. C. Chen, *Analyst*, 2008, **133**, 768.

2 W. W. Yu, L. H. Qu, W. Z. Guo and X. G. Peng, *Chem. Mater.*, 2003, **15**, 2854.

3 G. Frens, *Nat. Phys. Sci.*, 1973, **241**, 20.