Electronic Supplementary Information for the article **"SERS Opens a New Way in Aptasensor with High Sensitivity and Selectivity"** by Yuling Wang, Hui Wei, Bingling Li, Wen Ren, Shaojun Guo, Shaojun Dong* and Erkang Wang

Experimental details:

Materials: 6.5 μM ssDNA was prepared by 15-mer–α-thrombin DNA aptamer (5'-SH-$(CH_2)_6$-GGTTG GTGTG GTTGG-3') in 34 mM Tris-HCl buffer (pH=7.4, 233 mM NaCl, 8.5 mM KCl, 3.4 mM $MgCl_2$). Different concentrations of the α-thrombin and foreign proteins are all prepared in the Tris-HCl buffer.

Modification of the Au substrate by TBA and α-thrombin: Au substrate was first treated with a solution containing 6.5 μM of TBA in 34 mM Tris-HCl (pH=7.4) buffer containing 233 mM NaCl, 8.5 mM KCl, 3.4 mM $MgCl_2$ for 20 h. After washing with the Tris-HCl buffer, the DNA-functionalized Au-surface was treated with 2-mercaptoethanol (1×10^{-3} M in Tris-HCl buffer) for 1 h to yield the TBA assembly on the electrode surface. The resulting monolayer functionalized electrode was incubated 60 min with various concentrations of α-thrombin in Tris-HCl buffer for protein–aptamer interaction. The assembly processes were characterized by electrochemical measurements as shown in the Fig. S1.

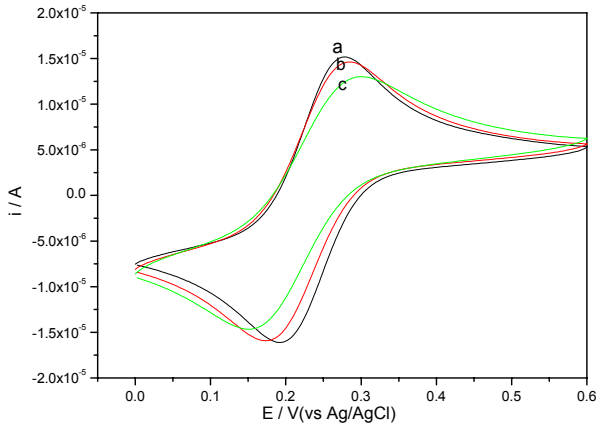

Fig. S1. Electrochemical cyclic voltammetry of bare Au (a), ME-TBA modified Au (b) and 15 nM α-thrombin modified Au(c).

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Synthesis and modification of Au NPs. Au-NPs stabilized with citrate were synthesized according to the literature procedure (J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc*. 1998, **120**, 1959). That, 100 mL of 1mM $HAuCl_4$ was brought to a reflux while stirring and then 10 mL of a 38.8 mM trisodium citrate solution was added quickly, which resulted in a color change of the solution from pale yellow to deep red. After the color change, the solution was refluxed for an additional 15 min. TEM image shows the diameter of such Au NPs obtained is ~13 nm and the UV-Vis spectrum shows the maximum extinction value of the 519 nm plasmon peak is ~3.0 shown in figure S2a and S3a.

R6G was chosen as the Raman reporter owing to its absorption maximum is tuned with the laser wavelength (514.5 nm) for added resonance enhancement and the clear vibrational features reported by many authors. (P. Hildebrandt, M. Stockburger *J. Phys. Chem.* 1984**, 88***,* 5935. M Campbell, S. Lecomte, W. E. Smith *J. Raman Spctrosc.* 1999, **30,** 37)

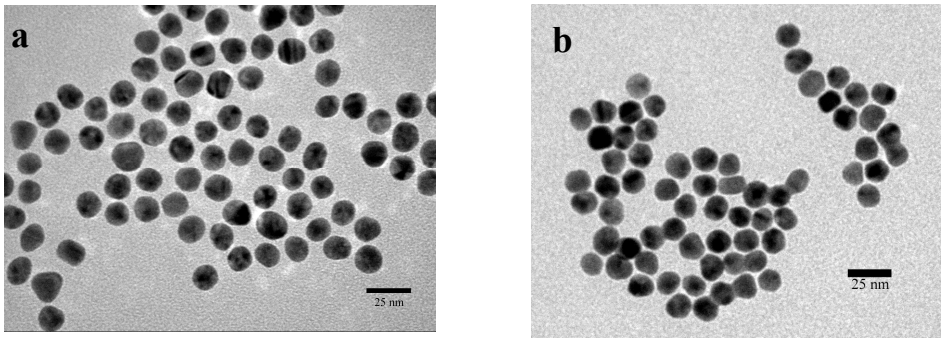

Fig. S2. TEM images of Au NPs (a) and TBA and Raman reporters modified Au NPs (b).

Au NPs modified by TBA and R6G were prepared according to the literature after a little modification. That, transfer 3 mL of the already prepared Au NPs to the NaOH-treated glass vials and then add 50 μL 6.5 μM TBA with magnetic stirring to facilitate the reaction for 16 h. The final Tris-HCl concentration is 0.56 mM. Add 18 μL of 10^{-4} M R6G to above vial with stirring for another 8 h and store the vial at 4° C for another day before use. Centrifuge the modified Au NPs at 14,000g at room temperature for 25 min twice to remove the free DNA and R6G. Again, disperse the

Au NPs in 2 mL of buffer containing 4.7 mM NaCl, 0.56 mM Tris-HCl, 0.14 mM KCl, pH=7.4.

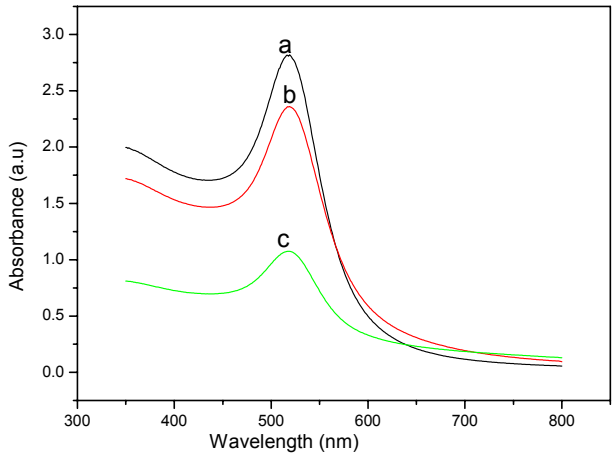

Fig. S3. UV-Vis absorption of AuNPs (a) and that modified by TBA and R6G before (a) and after centrifugation (c).

Figure S2b and Figure S3c give the TEM image and the UV-Vis absorption spectra of AuNPs modified by TBA and R6G after centrifugation, respectively, from which monodispersion of AuNPs and the surface plasmon absorption of AuNPs slightly shifted to 520 nm can be observed clearly. UV-Vis absorption spectra of AuNPs modified by TBA and R6G were also conducted before centrifugation as shown in curve b of figure S3, from which one can see that the addition of TBA and R6G into AuNPs stabilized by citrate did not change the absorbance of AuNPs at 519 nm greatly. After centrifugation, wider visible absorption peak appears at 520 nm (curve c) with a decreased intensity. The redshifting of surface-plasmon band with 1 nm can be attributed to the interparticles interactions caused by the aggregation of part gold nanoparticles. But this can not affect the background of SERS signal. Moreover, photographs of AuNPs stabilized by citrate, modified by TBA and R6G before and after centrifugation have been provided in Fig. S4, from which, it can be seen that AuNPs modified by TBA and R6G were not aggregated obviously.

In addition, the stability of the AuNPs modified by TBA and R6G has been investigated by UV-Vis absorption spectra as shown in figure S5. The standard error of the absorption intensity is lower than 6% within one week, indicating the stability of AuNPs modified by TBA and R6G before centrifugation can be retained at least

one week.

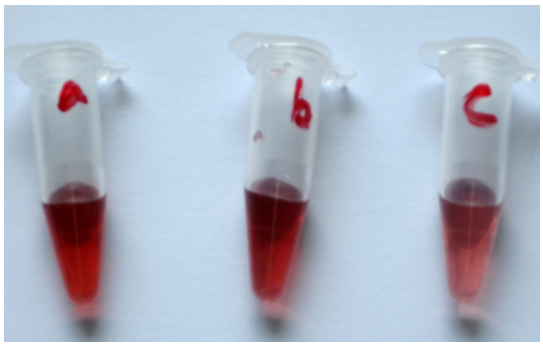

Figure S4. Photographs of 13 nm AuNPs stabilized by citrate (a), modified by TBA and R6G before (b) and after (c) centrifugation.

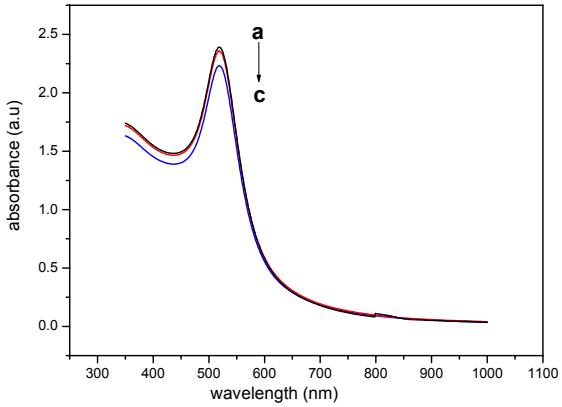

Figure S5. UV-Vis absorption of AuNPs modified by TBA and R6G with different days. a. o days, b. 4days and c. 7days before centrifugation.

Fabrication of sensing interface with a sandwich type system of TBA/α-thrombin/ TBA-Au nanoparticles (Au NPs). Au substrate immobilized by TBA and thrombin was immersed into the AuNPs modified by TBA and Raman reporter solution for 2 h to bind AuNPs modified by TBA and Raman reporters. Any unbound AuNPs were removed by firstly immersing the substrate into the buffer solution for eight minutes and washing with buffer and pure water for several times.

Preparation of Ag nanoparticles. Ag NPs were prepared by citrate reduction of $AgNO_3$ according to the literature (P.C. Lee, D. Meisel *J. Phys. Chem.* 1982, **86**, 3391). Briefly, silver nitrate (18mg) was suspended in 100 mL of pure water at 45° C and rapidly heated to boiling before 2 mL 1% solution of tri-sodium citrate was added under vigorous stirring. The solution was held at boiling for 90 min until continuous stirring upon cooling. The volume was adjusted to 100 mL with de-ionized water and

the concentration of silver colloids was 9.0×10^{-11} mol /L.

Electrochemical measurements: All electrochemical experiments were done in 5mM $Fe(CN)_6^{3-/4-}$ probe solution on an Autolab PGSTAT30 (Utrecht, The Netherlands) electrochemical system with a conventional three-electrode test cell. The cell was housed in a homemade Faraday cage to reduce stray electrical noise. Ag/AgCl (saturated KCl) electrode was used as the reference electrode, a Pt coil as the counter electrode, and gold disk as the working electrode.

TEM, SEM and UV-Vis spectroscopy: TEM and SEM images of Au NPs were conducted on the HITACHI H-8100 Transmission Electron Microscope operated at 200KV, and XL30 ESEM FEM Scanning Electron Microscope operated at 20KV, respectively. UV-Vis spectra of Au NPs were recorded using a CARY500 Scan UV-vis-NIR spectrophotometer.

SERS measurements: SERS spectra were recorded on a J-Y T64000 microRaman spectrometer (Jobin-Yvon, France) equipped with a liquid cooled charge-coupled device (CCD) detector. Spectra were excited using the 514.5 nm line of a continuous wave Ar ion laser (SpectraPhysics 2713). The incident laser beam was focused, and the signal was collected using a 50× objective. Approximately 5 mW of laser irradiation were used to excite the sample and the signal collection time was 30 s. And before each measurement, Raman spectrum of single crystal Si at 520 cm^{-1} is used to calibrate the spectrometer.

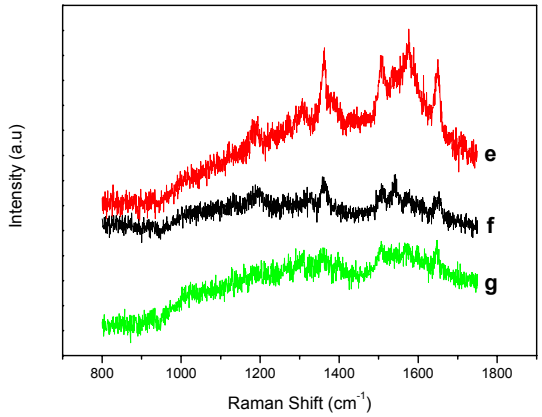

Fig. S6. SERS spectra of Raman reporters with different concentrations of α-thrombin: e. 5 nM, f. 0.5 nM and g. 0 nM.