Electronic Supplementary Information for the article **"Ultrasensitive Colorimetric Detection of Protein by Aptamer-Nanoparticles conjugates based on a Dot-blot Assay"** by Yuling Wang, Dan Li, Wen Ren, Zuojia Liu, Shaojun Dong* and Erkang Wang

Experimental details:

Materials: 15-mer–α-thrombin DNA aptamer, α-thrombin, β-, γ- thrombin and bovine serum albumin (BSA) were purchased from Aldrich. Silver enhancer kit (including solution A and solution B) was purchased from Sigma. Nitrocellulose membranes with porous diameter of 0.45 μm were purchased from BioRad.

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.

Labeling TBA with colloid AuNPs: Au nanoparticle (AuNPs) 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; M. C. Daniel, D. Astruc, *Chem. Rev.*, 2004, **104**, 293.). TEM image shows the diameter of such AuNPs 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 Fig. S1.

AuNPs labeled by TBA were prepared according to the literature after a little modification. That, transfer 3 mL of the already prepared AuNPs 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. The AuNPs were aging for 1 day and then centrifuge the modified AuNPs at 13,000g at room temperature for 15 min to remove the free DNA. Again, disperse the Au NPs in 8 mL of buffer containing 23.3 mM NaCl, 3.4 mM Tris-HCl and 0.34 mM $MgCl_2$, pH=7.4.

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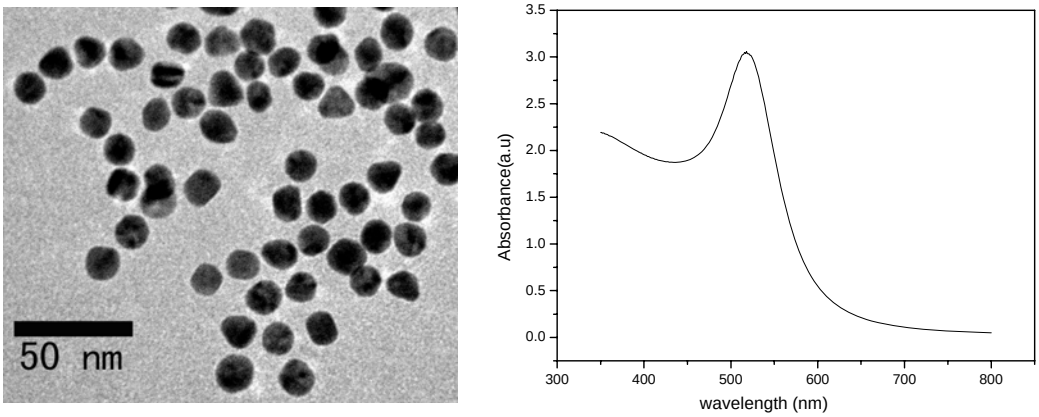

Figure S1. TEM image and UV-Vis absorption spectrum of Au NPs used in this communication.

Silver Enhancement Method. Silver enhancement reagent were used to intensify the signal of AuNPs in order to detect very low levels of protein. The above tested membranes were soaked in silver enhancer solutions for 8 min, which were mixed by solution A and solution B with the ratio of 1:1 immediately before use.

TEM and UV-Vis spectroscopy: TEM images of AuNPs were conducted on the HITACHI H-8100 Transmission Electron Microscope operated at 200 KV. UV-Vis spectra of AuNPs were recorded using a Cary 500 Scan UV-vis-NIR spectrophotometer.

Pretreatment of human plasma and spiked samples: Healthy human plasma was pretreated with the salt solution to avoid the formation of fibrin and the rapid sample clotting according to the procedure reported by Mascini and co-workers (Bini, A.; Minunni, M.; Tombelli, S.; Centi, S.; Mascini, M. *Anal. Chem.* **2007**, 79, 3016. Centi, S.; Tombelli, S.; Minunni, M.; Mascini, M. *Anal. Chem.* **2007**, 79, 1466.) That, 500 μL of plasma was treated with 2.5 mL of 2 M ammonium sulfate and 2.0 mL of 0.1M sodium chloride, which were mixed for 3-4 min, then centrifuged at 10000 rpm for 10 min and the supernatant was eluted by Tris-HCl buffer through gel column (Sephadex G-100) for rapid desalting and buffer exchange. Standard solutions of thrombin were spiked into the diluted pretreatment of 1% plasma to test the performance of the sensor in the complex matrix.

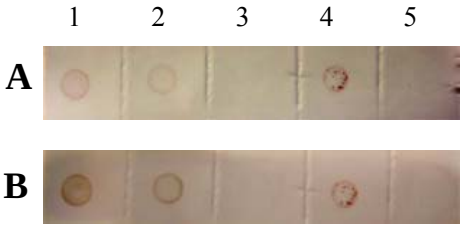

Figure S2. Detection of thrombin and control experiments without (A) and with silver enhancement (B). Red dot images for different levels of α-thrombin from 1 to 2 is 1.85, 0.925 pmole, respectively. Control experiments were conducted on 3, 4, 5 for 10 pmole of BSA, β-thrombin and γ-thrombin, respectively.

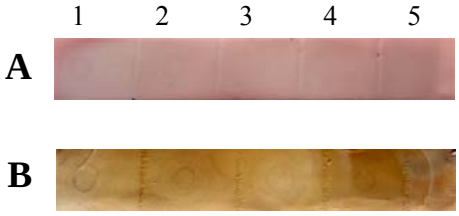

Figure S3. Detection of thrombin and control experiments without (A) and with silver enhancement (B). Red dot images for different levels of α-thrombin from 1 to 4 is 115 fmole, 57.5 fmole, 28 fmol and 14 fmole, respectively. Control experiments were conducted on 5 for 1.0 pmole of BSA.

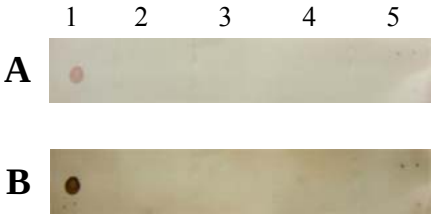

Figure S4. Detection of thrombin (spot 1, 9.25 pmole) and control experiments without (A) and with silver enhancement (B). Red dot images for different concentration of plasma from 2 to 5 is 1%, 2%, 5% and 8%, respectively.

Figure S5. Detection of thrombin (spot 1, 9.25 pmole) and control experiments without (A) and with silver enhancement (B). Red dot images for different concentration of BSA from 2 to 5 is 0.1%, 1%, 2% and 5%, respectively.