Electronic Supplementary Information for the article **"SERS Aptasensor from Nanorod-Nanoparticle Junction for Protein Detection"** by Yuling Wang, Kyuwan Lee and Joseph Irudayaraj*

Experimental details:

Materials: HPLC purified Thrombin binding aptamer (TBA) (5'-SH-$(CH_2)_6$-GGTTG GTGTG GTTGG-3') was purchased from IDT (Coralville, IA). Reductacryl was obtained from EMD Biosciences (San Diego, CA). 4-mercaptobenzoic acid (MBA), 4-mercaptopyridine (MPY), and Rhodamine B isothiocyanate (RITC) were purchased from Sigma-Aldrich (St. Louis, MO). Human α-Thrombin, BSA and IgG were obtained from Sigma-Aldrich. Sheep polyclonal antibody to thrombin was obtained from ABR-Affinity Bioreagents (Thermo Scientific Company). 34 mM Tris-HCl buffer solution (pH=7.4) with 233 mM NaCl, 8.5 mM KCl, 3.4 mM $MgCl_2$) were prepared for all experiments.

Reduction of Disulfide Bond: Thiolated TBA obtained from IDT in a disulfide form was reduced using dithiothreitol immobilized onto acrylamide resin (Reductacryl) according to a protocol provided by IDT. The oligo and Reductacryl were first resuspended in water at a ratio of 1 mg of oligo to 50 mg of resin to ensure complete reduction, and the mixture was stirred at room temperature for 15 min. Reductacryl was finally removed by syringe filtration (pore size 0.2 μm).

Synthesis and modification of AuNPs by TBA and Raman Reporter: AuNPs stabilized with citrate were synthesized according to the procedure reported in the literature (J. J. Storhoff, R. Elghanian, R. C. Mucic, C. A. Mirkin and R. L. Letsinger, *J. Am. Chem. Soc*. 1998, **120**, 1959). Here, 100 mL of 1mM $HAuCl_4$ was brought to a reflux while stirring and 10 mL of a 38.8 mM trisodium citrate solution was added instantly to result 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.

Mercaptobenzoic acid (MBA), mercaptopyridince (MPY), RITC were chosen as the Raman reporter because of their distinct vibrational features reported by many

authors. (S. Xu, X. Ji, W. Xu, X. Li, L. Wang, Y.i Bai, B. Zhao and Y. Ozaki, *Analyst.* 2004**, 129**, 63; C. J. Orendorff, A. G. Tapan, K. Sau, C. J. Murphy, *Anal. Chem.,* 2005, **77**, 3261; J. Zhou, S. Xu, W. Xu, B. Zhao, Y. Ozaki, *J.Raman Spectrosc*., 2009, **40**, 31; K. Kim, H. B. Lee, Y. M. Lee, K. S. Shin, Biosensors and Bioelectronics, 2009, 24, 1864)

AuNPs modified by TBA and Raman Reporters were prepared according to the literature after minor modification. Here 1.5 ml of the as prepared AuNPs was transferred to NaOH-treated glass vials and 50 μL of 13.35 μM of TBA was added with stirring to facilitate the reaction for 16 h. Then 18 μL of 10^{-4} M Raman Reporters (10^{-6} M RITC) was added to the above vial and stirred for another 8 h and the resulting solution was stored in a vial at 4° C for one day before use. The modified AuNPs was centrifuged at 13,000 rpm for 15 min in room temperature twice to remove free DNA and Raman reporters. AuNPs were then dispersed in 1 mL of buffer containing 4.7 mM NaCl, 0.56 mM Tris-HCl, 0.14 mM KCl, pH=7.4.

Synthesis and modification of AuNRs by anti-thrombin antibody. CTAB-stabilized AuNRs were synthesized using the seed-mediated growth method improved by El-Sayed and co-workers. (B. Nikoobakht, M. A. El-Sayed, *Chem. Mater.* 2003, **15**, 1957). Briefly, the seed solution was prepared by mixing CTAB (0.2 M, 5 mL) and $HAuCl_4$ (0.5 mM, 5 mL) with freshly prepared ice-cold $NaBH_4$ (10 mM, 0.6 mL). After 3 h, this seed solution was used for the synthesis of AuNRs. 20.0 mL of 0.2 M CTAB was mixed with 400 μL of 10 mM silver nitrate and 20.0 mL of 1 mM $HAuCl_4$. After gently mixing the solution, 220 μL of 0.10 M ascorbic acid was added. Then, 48 μL of the seed solution was finally added to the mixture to initiate growth to yield AuNRs of aspect ratio ~ 3.9. Excess CTAB was removed by centrifuging twice at 8000 rpm, and the supernatant disregarded and the resulting particles redispersed in pure water.

Modification of AuNRs was accomplished according the method previously reported by our group (C.Wang, J. Irudayaraj, *Small,* 2008, **4**, 2204). Here, 0.1 mL of 30 mM aqueous solution of cystamine was added into 1 mL of AuNRs solution and

centrifuged once and sonicated for 3 h at 50 ºC. The resulting AuNRs were then collected by centrifuging twice at 7000 rpm for 15 min to remove excess cystamine and CTAB and resuspended in a 0.005 M CTAB solution to yield a final concentration of 100 nM. Then 0.1 mg mPEG-SH (MW 5000) was added to 1 mL of amine group bearing AuNRs. The solution was mixed and stirred for 3 h at room temperature, and centrifuged at 7000 rpm for 10 min.

Immobilization of anti-thrombin antibodies onto the amine functionalized AuNRs was accomplished using the well-established glutaraldehyde spacer method; 1 mL of amine-functionalized nanoparticles was dispersed into 0.01 M PBS (pH 7.4) containing 5% glutaraldehyde for about 1 h. These particles were then collected by centrifugation and redispersed in PBS, and incubated with the antibody for 12 h at 4°C. The antibody-modified AuNRs were washed with PBS to remove excess antibody and kept at 4 °C in pH 7.4 PBS.

Fabrication of AuNRs/α-thrombin/ TBA-AuNPs junction sensor interface. 200 μL of AuNRs immobilized with anti-thrombin antibody were first mixed with 100 μL AuNPs modified by TBA and Raman reporters and 100 μL of different concentrations of human α-thrombin was added to the above solution to assemble AuNPs onto AuNRs due to the formation of the antibody-protein-aptamer complex. The resulting solution was reacted at 28°C under string for 2 h and any unbound AuNPs were removed by centrifugation at 4000 rpm for 10 min.

TEM and UV-Vis spectroscopy: The aspect ratios of AuNRs were determined from transmission electron microscopy (TEM) images, acquired with a Philips CM-100 TEM (Philips, Eindhoven, Netherlands) operating at 100 kV. UV-Vis absorption spectra of all samples at every step of the experiments were measured with a Jasco V570 UV/visible/NIR spectrophotometer (Jasco, Inc., Easton, MD), in the 400 and 1100 nm wavelength range.

SERS measurements: SERS spectra were recorded using the Bruker microRaman spectrometer and excited using the 633 nm line. The incident laser beam was focused, and the signal collected using a 50× long distance objective. Approximately 20 mW of

laser irradiation were used to excite the sample for a signal collection time of 20 s. Before each measurement, the spectrometer was calibrated using the Raman spectrum of a single Si crystal using the distinct peak at 520 cm^{-1}.

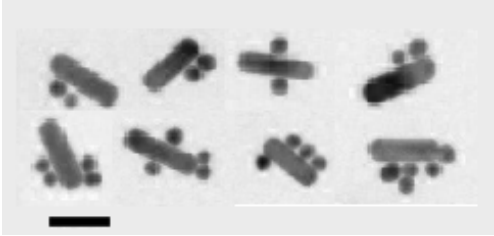

Fig. S1. Additional TEM images of AuNR-AuNPs junction showing the binding of AuNPs onto AuNRs through a thrombin sandwich assay (The scar bar is 50 nm).

Finite Difference Time Domain (FDTD) simulation: A commercial FDTD package (RemCom SFDTD 6.2) employing the Lorentz-Drude model was used for simulating the electromagnetic field enhancement in the vicinity of the nanoparticle structure in vacuum. The structure of nanoparticles used for simulation constitutes a 13×50 nm rod associated with 13 nm spheres with the gap of 2 nm in between nanoparticles. The local electromagnetic field excited by 633 nm laser show a maximum enhancement of ~10^5 between AuNPs-AuNR junction with the vertical orientation (Fig.S2A) and ~10^7 with the oblique orientation (Fig. S2B). Thus very sensitive devices can be constructed using these structures.

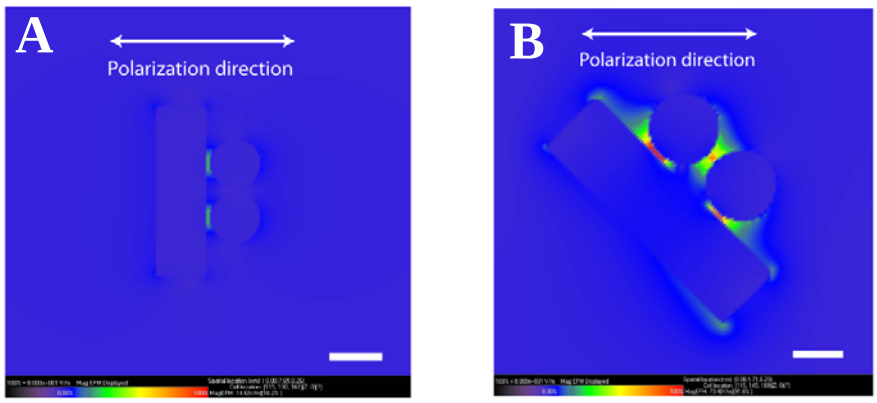

Fig. S2. Electromagnetic near field enhancement calculated by FDTD method with an excitation laser wavelength of 632.8 nm to simulate (A) the vertical AuNPs-AuNR junction, (B) the oblique AuNPs-AuNR junction. Scale bar is 10 nm.

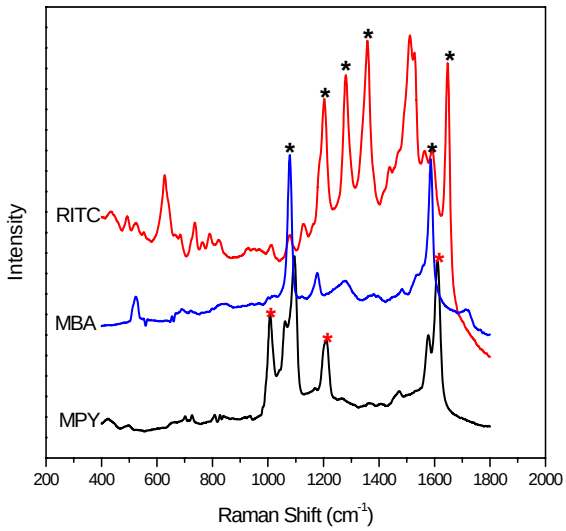

Fig. S3. SERS spectra from three different probes each with a unique Raman label for TB detection (at 8.7 nM concentration) shows the possibility of multiplex protein detection.