Electronic Supplementary Information for the article **"A SERS DNAzyme Biosensor for Lead Ion Detection"** by Yuling Wang and Joseph Irudayaraj*

Experimental details:

Materials*:* All DNA oligonucleotides with HPLC purification were purchased from IDT (Coralville, IA). The sequences of the DNA oligonucleotides employed in this work are given below:

DNAzyme: 5'-HS-$(CH_2)_6$-TTT TTC ATC TCT TCT CCG AGC CGG TCG AAA TAG TGA GT-3'

Substrate: 5'-TGT CAA CTC GTG ACT CAC TAT rAGG AAG AGA TG-3'

DNA sequence for AuNPs conjugating: 5'-HS-(CH2)6-CAC GAG TTG ACA-3'

The Reductacryl was obtained from EMD Biosciences (San Diego, CA). HEPES, 6-mercapto-1-hexane, and 4-mercaptobenzoic acid were purchased from Sigma-Aldrich (St. Louis, MO).

Reduction of Disulfide Bond**:** Thiolated DNA oligonucleotides obtained from IDT in a disulfide form were 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).

Preparation of AuNPs and oligo-modified AuNPs: AuNPs stabilized with citrate were prepared according to the literature reported. In briefly, 100 mL 0.01% $HAuCl_4$ solution were heated to boiling and then 1mL 1% sodium citrate was added and boiling for another 5 min. The size of gold nanoparticles is about 40 nm according to the TEM image.

Thiol-derivated single-stranded oligonucleotide after reduction of disulfide bond was bound to AuNPs directly using the procedure developed by Demers et al and our group with slight modifications. Thiol-modified DNA oligonucleotides were added into 1 mL of AuNPs solution to result in a final concentration of 1 μM. After 24 h, the Raman reporters (MBA solution with a final concentration of 25μM) were added into the AuNPs

solution for another 24 h. After that, the AuNP conjugates labeled by DNA and Raman reporters were "aged" in the solution (0.3 M NaCl, 25 mM HEPES, pH 7.04) for another 48 h. Excess reagents were removed by centrifuging at 6000 rpm for 10 min. The red oily precipitate was washed, recentrifuged, and then dispersed in 25 mM HEPES buffer including 100 mM NaCl (pH 7.04).

Sensor Preparation: The gold-coated glass slide was first treated with piranha solution (3:1 concentrated sulfuric acid to hydrogen peroxide solution) for 1 h to clean off the organic residue and to produce a hydrophilic surface. The cleaned gold surfaces were interacted with a solution composed of 1 μM DNAzyme (1), 25 mM HEPES (pH 8.2) for 16 h at room temperature. The surfaces were then passivated with 1 mM MCH for 60 min. The resulting DNAzyme monolayer functionalized surfaces were treated with the mixture of substrate strand and the above DNA-AuNP conjugates, 2.0 μM, in 25 mM HEPES (pH 7.04) including 100 mM NaCl in a 80 °C water bath for 10 min. The water bath was then allowed to cool to room temperature over 60 min. The hybridization lasted 2 h at room temperature. After incubation in buffers at 37 °C for 1h to reduce the physical adsorption, the ds-DNA surfaces were then allowed to react with various concentrations of target Pb^{2+} in buffers (25 mM HEPES + 0.1 M NaCl, pH 7.04) for 60 min in a 37 °C water bath to obtain the maximum cleavage of the substrate strand on the gold surface. After that, the gold surface were washed three times using the buffer and water in turn.

TEM, SEM and UV-Vis spectroscopy: The morphology and the size of AuNPs 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 900 nm wavelength range. Surface image of AuNPs on the gold surface were performed using Field-emission scanning electron microscopy (SEM) images, obtained with a NOVA nanoSEM FESEM.

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 was 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. A

50 µm pinhole was used for confocal purpose and the resolution was set to 3 to 5 cm^{-1}. Before each measurement, the spectrometer was calibrated using the Raman spectrum of a single Si crystal using the distinct peak at 520 cm^{-1}

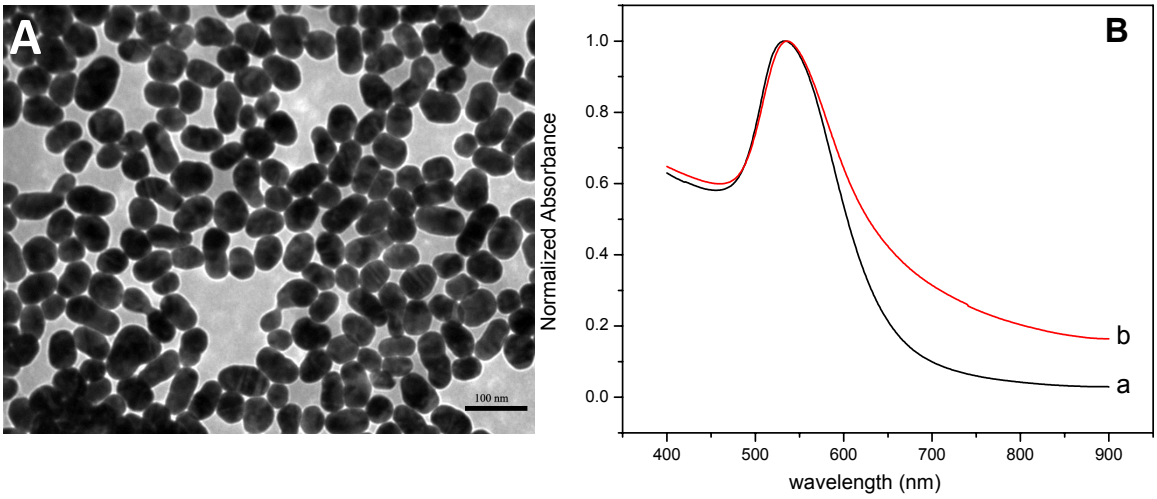

Fig. S1. TEM image of AuNPs (A) and UV-Vis absorption spectra (B) of AuNPs (a), AuNPs labeled by DNA and Raman Reporters (b).

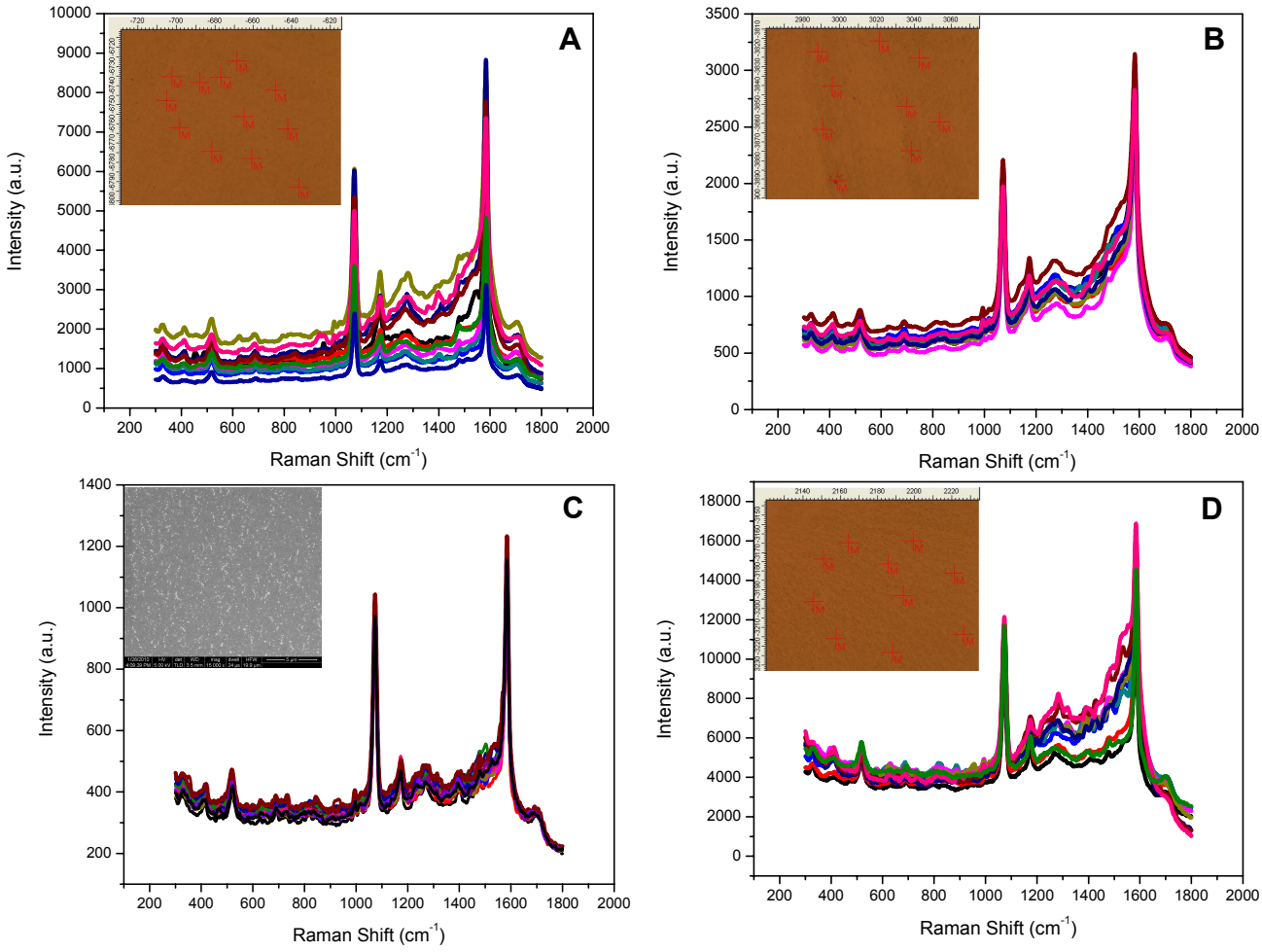

Fig. S2. Replicate SERS spectra of Raman reporters for Pb^{2+} ions detection at 20 nM (A) 50 nM (B), 500 nM (C) and control experiment for Zn^{2+} (D) to show the reproducibility of the designed SERS DNAzyme biosensor for Pb^{2+} ions detection. Inset picture in A, B and D shows the optical image of the gold surface and the points selected for Raman measurement. Inset picture in C shows the SEM image of gold surface after the catalytic reaction of Pb^{2+} ions with DNAzyme.