Electronic Supplementary Information

An Immunoassay Using Biotinylated Single Walled Carbon Nanotubes as Raman Biomarkers

Chengfan Cao, Jung Heon Kim, Ye-Jin Kwon, Young-Jin Kim, Eung-Soo Hwang,* and Seunghyun Baik*

I. Experimental Procedures

1. Preparation of SWNT-ssDNA-biotin probe

The sequence of oligonucleotide (Geno Technology) is 5'-d$(GT)_{15}$-3', and the 3' end is pre-modified with biotin. The SWNT-ssDNA-biotin probes were prepared utilizing a similar protocol developed by Zheng et al..[1] Biotin-conjugated ssDNA was mixed with HiPco SWNTs (Carbon Nanotechnology, Inc.) at a 1:1 mass ratio in 0.15 M NaCl in distilled water. The tube diameters of HiPco SWNTs are between 0.7 and 1.1 nm, and an average length is about 1 μm. In the next step, the mixture was sonicated for 3 hours at room temperature. The sample was subsequently centrifuged for 3 hours at 13,000 g and the pellet was discarded. Finally, free biotin-ssDNA in the supernatant, which is not conjugated with SWNTs, was removed by a dialysis technique using Spectra/Por Biotech CE membranes (100 kDa MWCO) in a standard TRIS buffered solution for 2 days. The nanotube concentration was about 20 mg/L.

2. Incubation of HFLFs

HFLFs were grown in Dulbeccos' modified Eagle's medium (Gibco) supplemented with 10% FBS(JRH), 1 X amphotericin B (Gibco) and 1 X penicillin-streptomycin (Gibco) in a humidified 5% CO_2 incubator at 37 ℃. HFLF was infected with HCMV at 1 m.o.i. for 90 min in the CO_2 incubator.

II. Dot blotting: targeting SCMVM34 using the SWNT-ssDNA-biotin probe

1. One-step reaction

The reaction between SWNT-ssDNA-biotin and SCMVM34 was carried out in one-step using a protocol described in the main text of paper. Avidin and biotin-conjugated amtimouse IgG were purchased from Sigma, and SCMVM34 was prepared in the Lab.[2] Figure S1a shows Raman spectra from the nylon membrane after the dot blotting procedure (Kaiser Optical RXN1, 785 nm excitation). It shows Raman features related with nanotubes as well as other peaks attributed to the chemical compositions on the nylon membrane.[3] The G mode region of nanotubes is magnified in Figure S1b. Figure S1b is also provided in the main text of paper.

2. Multi-step reaction

Six different concentrations of SCMVM34 were dotted on the nylon membrane as described in the previous section. The nylon membrane was then treated in skim milk (2 wt%) followed by 3 times of 15 min washing with PBS-T buffer. In the next step, biotin-conjugated anti-mouse IgG was reacted with SCMVM34 on the nylon membrane for 1 hour followed by washing (PBS-T, 3 times of 15 min). Avidin was reacted for 1 hour, and the nylon membrane was washed again (PBS-T, 3 times of 15 min). Finally, the membrane was reacted with SWNT-ssDNA-biotin for 1 hour. The membrane was thoroughly washed before the Raman measurements. Figure S1c shows Raman spectra from the nylon membrane, and the G mode is magnified in Figure S1d. The intensity of the G mode increased as the concentration of SCMVM34 increased. However, the control spot did not show any signals related with nanotubes.

The area under the G mode was integrated from 1585 to 1615 cm^{-1} as shown in Figure S2. The intensity of Raman G mode increased as the concentrations of SCMVM34 increased in both methods. The intensities of the multi-step reaction were greater than those of the one-step conjugation. This could be related to the inefficient bridging between biotinylated SWNT and biotin-conjugated antimouse IgG with

avidin rather than the theoretical 2:1:2 molar bindings. The other possibility is the mass of the probe in the final washing stage. The mass of the SWNT-ssDNA-biotin-avidin-biotin conjugated antimouse IgG is greater than that of SWNT-ssDNA-biotin. Therefore, it is possible that some of the SWNT-ssDNA-biotin-avidin-biotin conjugated antimouse IgG complex is detached from the target at the final washing stage.

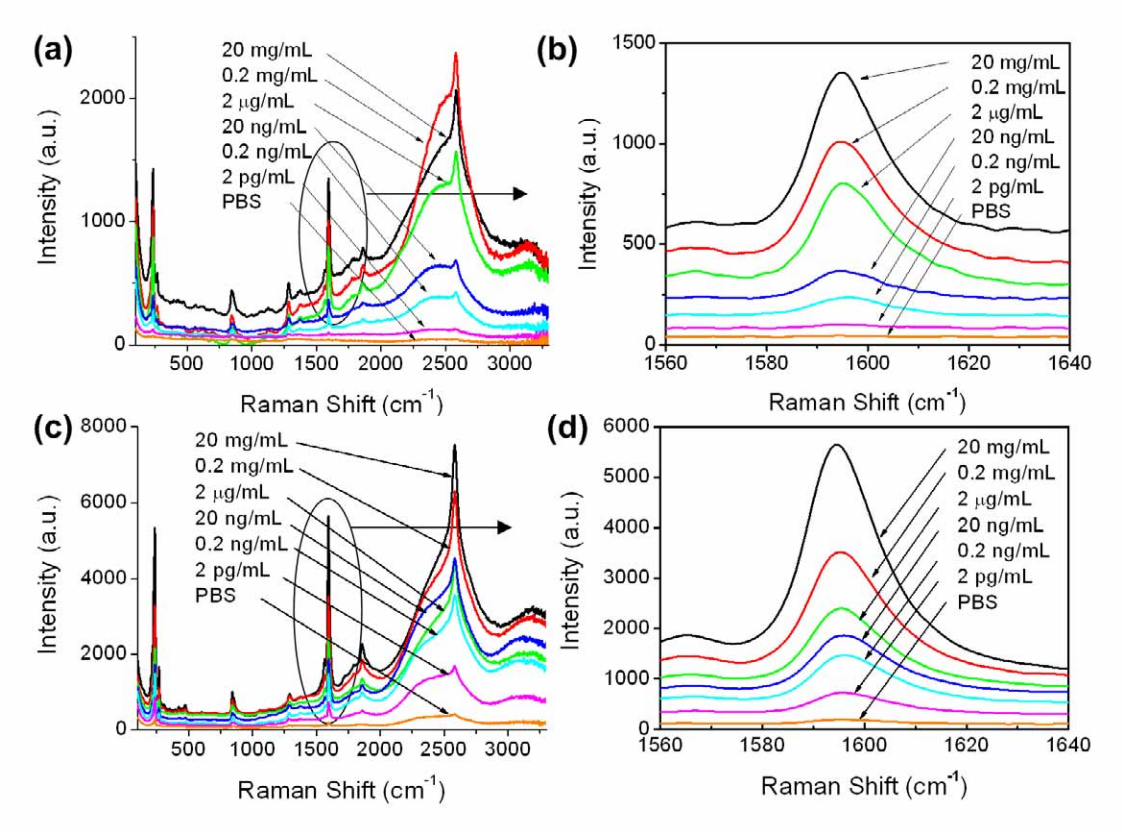

Figure S1. Raman spectra from the nylon membrane after the dot blotting procedure. (a) Raman spectra from the one-step conjugation (b) magnified G mode (one-step) (c) Raman spectra from the multi-step conjugation (d) magnified G mode (multi-step)

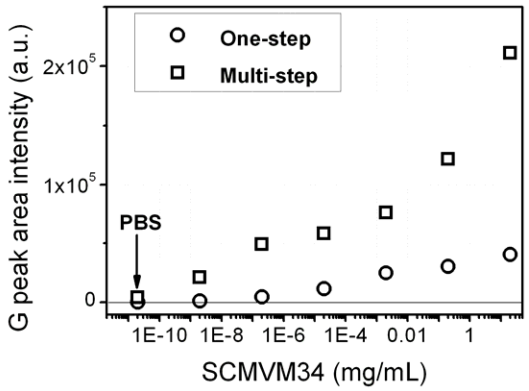

Figure S2. Integrated area intensities of the G mode..

III. Probing UL44 in HCMV-infected HFLF using the SWNT-ssDNA-biotin-avidin-biotin conjugated antimouse IgG and specific monoclonal antibody

Figure S3a shows Raman spectra from the nylon membrane after the dot blotting process The G mode is magnified in Fig. S3b, which is also provided in the main text of the paper.

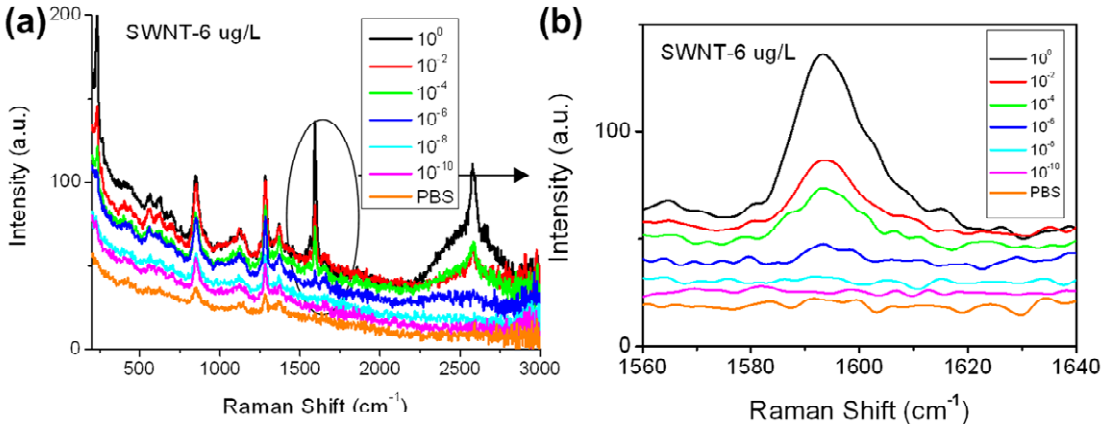

Figure S3. Raman spectra from the nylon membrane after the dot blotting process (a) Raman spectra (b) magnified G mode

IV. Photostable Raman Signal

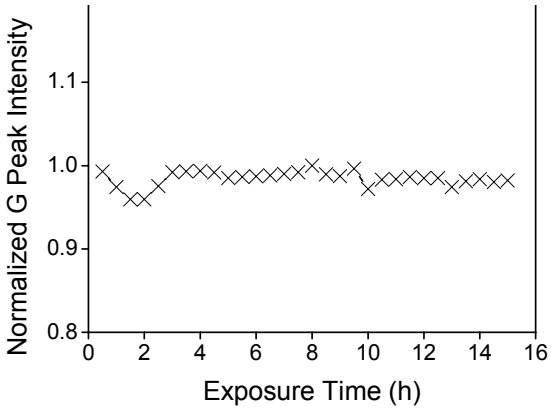

Figure. S4 Photostable Raman signals of nanotube probes conjugated with SCMVM34 on the nylon membrane. The power of the laser was 20 mW (785 nm excitation) and the spot size was 1~2 μm in diameter.

Figure S4 shows photostable Raman signals of nanotube probes conjugated with SCMVM34 on the nylon membrane. Repeated Raman measurements presented the strong resistance to photo-bleaching as previously reported.[3,4] The measurement was carried out every 30 minutes up to 15 hours. The exposure duration was 3 seconds each time. A slight variation in the G mode intensity could be due to the different focusing effect. Any noticeable degradation of the sample was not observed under the current experimental conditions.

References

(1) M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. Mclean, S. R. Lustig, R. E. Richardson and N. G. Tassi, *Nature mater*. 2003, **2**, 338.
(2) E. S. Hwang, J. Kim, H. S. Jong, J. W. Park, C. G. Park, C. Y. Cha, *Microbiol. Immunol.* **2000**, *44*, 827-832.
(3) E. S. Hwang, C. Cao, S. Hong, H. J. Jung, C. Y. Cha, J. B. Choi, Y. J. Kim, S. Baik, *Nanotechnol*. **2006**, *17*, 3442-3445.
(4) D. A. Heller, S. Baik, T. E. Eurel and M. S. Strano, *Adv. Mater.* 2005, **17**, 2793..