Electronic Supplementary Material (ESI) for Chemical Communications

Electronic Supplementary Information (ESI)

High-sensitive detection of self-aggregated single-walled carbon nanotubes using DNA-immobilized resonator

*Kuewhan Jang, Jinsung Park, Doyeon Bang, Sangmyung Lee, Juneseok You, Seungjoo Haam and Sungsoo Na**

Department of Mechanical Engineering, Korea University Seoul 136-701, Republic of Korea
Tel/ +82-2-32903854; E-mail: nass@korea.ac.kr

Experimental Section

Materials

The following materials were purchased from Sigma-Aldrich: sulfuric acid (H_2SO_4), hydrogen peroxide solution (H_2O_2), (3-aminopropyl)triethoxysilane (APTES), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide (EDC), N-hydroxysuccinimide (NHS), succinic anhydride (SA), magnesium acetate solution, dimethyl sulfoxide (DMSO), triethylamine (TEA), and tris-EDTA buffer solution. The following DNAs were purchased from Integrated DNA Technology, CA, USA: 5′-TTT TTT TTT TTT TTT TTT TTT TTT TTT GTT GCG AGG TCT TGC CGA CA-3′ (l1-DNA), 5′-TTT TTT TTC GTT GTC GGC AAG ACC TCG CAA C-3′ (l2-DNA), 5′-/5AmMC6/TGT CGG CAA GAC CTC GCA AC-3′ (c-DNA) and 5′-/5AmMC6/TGT CGG CAA GAC CTC GCA AC/36-FAM/-3′ (FITC-c-DNA).

Synthesis of self-crosslinked SWNT

SCLS was formed by conjugating DNA linkers with SWNTs, similar to a previous report.[1] First, l1-DNA and l2-DNA linker strands were added to a ~33-μM concentration in 5 ml of 1xTAE Mg buffer (10 mM tris acetate, 1 mM EDTA, 12.5 mM magnesium acetate). When two strands were used for the

linker, l1-DNA strands were added at a 10% excess to the l2-DNA strands. After incubation for 3 h, 5 mg of SWNT was added to the solution. The mixture was kept in an ice-water bath and sonicated for 90 min at a power level of 10 W.

Confirmation of self-crosslinked SWNT using AFM

For the morphology analysis of the SCLS, 2 μl of the SCLS solution was dropped on a silicon wafer. Then, the wafer was washed with deionized (DI) water and dried in a dessicator for a day. The SCLS morphology was obtained using the tapping mode of an Innova (Bruker, Santa Barbara, USA) with a nanodrive controller (Bruker, Santa Barbara, USA) in air at ambient temperature and pressure. A closed-loop scanner was used for the tapping-mode image, which allows one to obtain precise and reproducible images of each state. A commercial cantilever (SSS-NCHR, Nanosensors, Switzerland) was used for recording all of the images. The resonant frequency of the cantilever was 320 kHz, and the tip radius was ~2 nm. All of the images were scanned at 1 Hz. All of the images were leveled in two dimensions and processed using SPM Lab Analysis software V7.00 (Bruker, Santa Barbara, USA).

Synthesis of c-DNA-immobilized resonator

For CDR, we used a resonator (TESP, Bruker, Madison, WI, USA) having the dimensions of 40 × 4 × 125 μm3 (width × thickness × length). Amine-functionalized c-DNA was immobilized on a silicon-based resonator similar to previous report.[2] The resonator was first washed with DI water and dried in a dessicator for a day. Then, the resonator was immersed in piranha solution (3:1 of H_2SO_4 and H_2O_2) for 5 min, washed with DI water several times, and dried in a dessicator. After that, the resonator was immersed in a 10% APTES solution in EtOH for 5 min and rinsed with EtOH and DI water. The amino groups of APTES were converted to carboxyl groups by immersing in DMSO with 10 mg ml-1 of succinic anhydride (SA) and 1 mg ml-1 of triethylamine (TEA). After the reaction, the resonator was rinsed with DI water and dried in the dessicator.

Preparation of fluorescence experiment

We immobilized the FITC-c-DNA (5′-/5AmMC6/TGT CGG CAA GAC CTC GCA AC/36-FAM/-3′) on the resonator. The resonator was TESP (Bruker, Madison, WI, USA) and the DNA sequence was similar to that of the c-DNA, except for the fluorescence dye. The rest of the process was the same as that for the CDR synthesis. After attaching FITC-c-DNA on the resonator, we obtained the fluorescence image using a fluorescence microscope.

SWNT detection assay

For sensitivity detection, we prepared SCLS solutions with various concentrations by varying the SWNT concentration (1 mg ml^{-1}, 10 μg ml^{-1}, 100 ng ml^{-1}, 50 ng ml^{-1}, 10 ng ml^{-1}, 5 ng ml^{-1} and 1 ng ml^{-1}). After immersing the CDR in the solution for a day, it was washed with DMSO because DMSO is a well-known scavenger of OH.[3] Then, CDR was dried in the dessicator for a day, and the resonant frequency was measured using AFM (Bruker, Santa Barbara, USA). For a control experiment, the resonant frequency of the DNA linkers (l1-DNA and l2-DNA) without SWNTs was measured.

Raman spectroscopy

For Raman spectroscopy, we prepared a sample of CDR after immersion in each concentration of SCLS solution. Raman spectroscopy was performed using 514-nm laser excitation in a Renishaw system with a microscope. Spectra were collected from 100 to 3000 cm^{-1}.

SWNT detection in real tap water

To determine the detection performance in a practical sample, general tap water was used (Korea University, Seoul, Korea). The synthesis of SCLS was similar to that in the buffer solution except that tap water was used instead of the buffer solution. After the SCLS production in tap water, CDR was

immersed in solutions with SWNT concentrations of 10 μg ml^{-1}, 100 ng ml^{-1} and 10 ng ml^{-1}. The assay protocol was the same as that mentioned above for the SWNT detection assay.

Verification of DNA immobilization on resonator

In order to verify the c-DNA immobilization on the resonator, we employed fluorescein isothiocyanate (FITC) tagged c-DNA (FITC-c-DNA) on the surface of resonator. As shown in Fig. S1 in ESI†, we obtained optical and fluorescence images of bare, carboxyl group-modified, c-DNA immobilized, and SCLS solution (1 mg ml^{-1}) immersed resonators. From the optical images, we observed similar morphologies and could not see any particular differences between the each state. However, in the fluorescence images, we were able to see significant fluorescence only for the CDR and SCLS solution immersed resonator. In these resonators, green fluorescence was observed on the entire surface of the resonator, which implied that the c-DNA was evenly immobilized on the resonator and the resonator was able to capture the target SCLS. Moreover, the fluorescence of the SCLS immersed resonator was similar to that of the CDR, indicating that c-DNA effectively remained on the resonator even after immersion in the SCLS solution and attached c-DNA was enough to detect the SCLS which the weight was heavier than its weight.

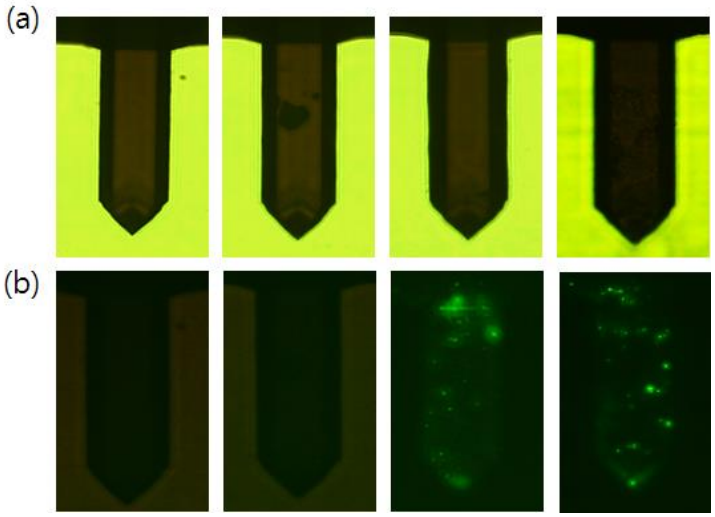

Fig. S1 (a) Optical images and (b) fluorescence images of bare, carboxyl group functionalized, c-DNA immobilized and self-crosslinked SWNT captured resonators

Verification of self crosslinked SWNTs

In order to investigate the shape of SCLS and the number of SWNTs in SCLS, we deposited a SCLS solution on a silicon wafer and obtained the morphology of SCLS using atomic force microscopy (AFM). An AFM image showed typical SCLSs maintaining a twig-like structure (Fig. S2). For further verification, we evaluated ~100 SCLSs and measured the number of SWNTs in each SCLS structure. The average number of SWNTs in a SCLS was 4.00 ± 1.68. This result implies that the SCLS conjugation induced a mass amplification that was at least 4 times of a single SWNT as a result of an increase in the number of SWNTs and the addition of DNA linkers in the SCLS. In addition, SCLS had advantage such as a constant number of SWNT in the SCLS where SWNT bundle aggregate rashly. In the SCLS solution, we also observed a small number of bundled SWNTs and un-conjugated SWNTs (data not shown), and this result might have been induced by the dispersion limit of the SWNT and DNA solution[4] and the binding of the unhybridized DNA linker only to the SWNT sidewall.[5]

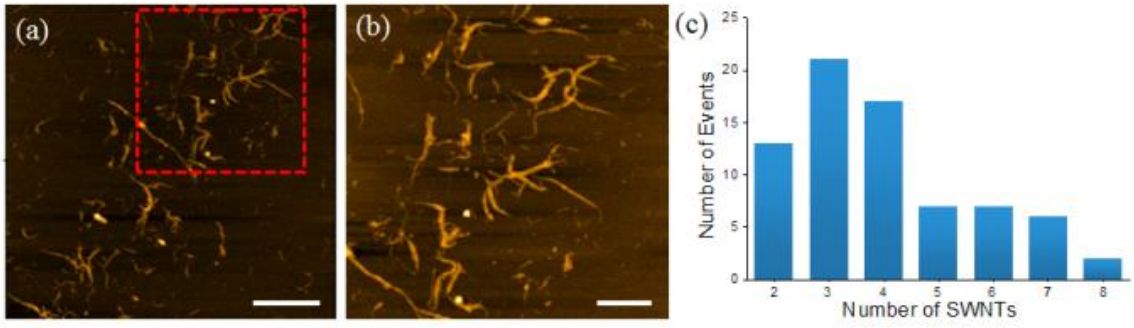

Fig. S2 Tapping-mode AFM image of (a) self-crosslinked SWNTs and (b) enlarged image of (a). The scale bar is 1 μm and 0.6 μm, respectively. (c) Histogram of a number of SWNTs in each self-crosslinked SWNT.

Verification of SWNT Detection

To verify the detection of SWNTs on the resonator surface, we employed Raman spectroscopy, which is a representative equipment of the SWNT verification. As shown in Fig. S3 (ESI†), Raman spectra of the resonator for concentrations of 1 mg ml^{-1}, 10 μg ml^{-1}, 100 ng ml^{-1}, and 1 ng ml^{-1} were shown, respectively. The Raman spectra showed typical SWNT features only at the 1 mg ml^{-1} and 10 μg ml^{-1} concentrations. Specifically, we observed the G band at 1591 cm^{-1}, D band at 1340 cm^{-1}, and 2D band at 2680 cm^{-1}.[6] However, the Raman spectra of the 100 ng ml^{-1} and 1 ng ml^{-1} concentrations was not

able to show any specific SWNT bands, which might be a result of the limitation of the Raman spectroscopy sensitivity.[7] From all of the samples, we observed a peak at around 520 cm^{-1}, and this peak corresponded to the peak of the silicon from the silicon wafer.[8] Generally, Raman sensor is only able to detect the sample within the beam diameter of 1μm^2. The probability of SWNT absorption on the surface will decrease exponentially at low concentrations such as 100 ng/ml and 1 ng/ml. From this reason, there is no Raman signal 100 and 1 ng/ml.

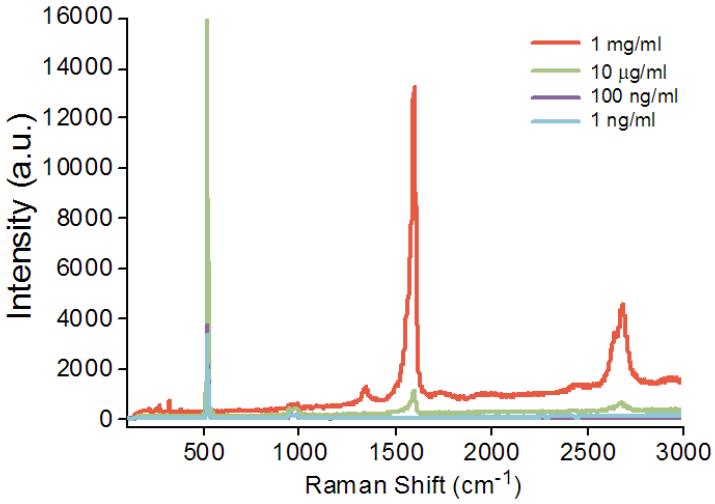

Fig. S3 Raman spectra of the self-crosslinked SWNT detected resonators under various concentration of 1 mg ml^{-1}, 10 µg ml^{-1}, 100 ng ml^{-1} and 1 ng ml^{-1}.

Non-specific binding with metal ions

Tap water contains various metal ions and those ions might non-specifically bind to the surface of the resonator. From this reason, we have checked the non-specific interaction with other ions. Fig. S4 (ESI†) shows the resonant frequency shift of resonator in various ions (Ca^{2+}, Fe^{3+}, Na^{+} and Zn^{2+}) dissolved in the buffer solution, respectively. All ions and SWNT concentration were equal (10 µg/ml). Here, the percentage of the metal ion reactivity is described as $\%N = \omega_m(\text{metal ion})/\omega_s(10\ \mu g/ml\ \text{of SWNT})$. We notice that ions interaction are negligible comparing to SWNT detection.

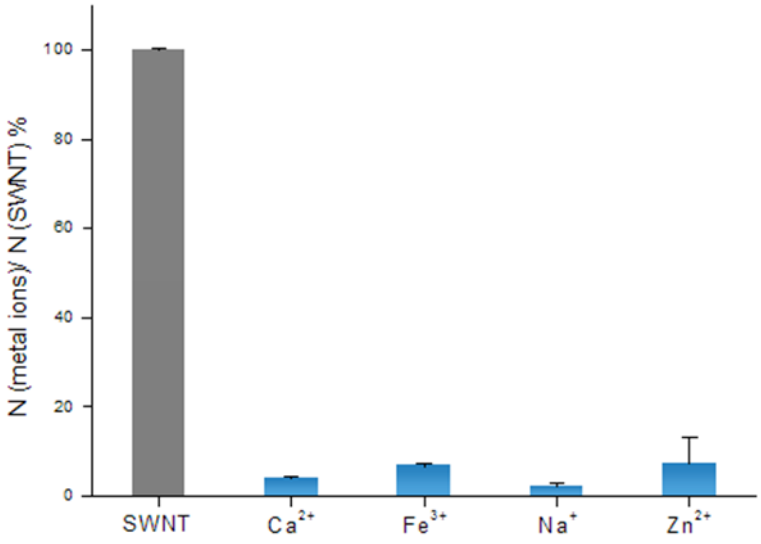

Fig. S4 Analysis of non-specific interaction of tap water. The concentration of SWNT and all other interfering metal ions (blue bar) were 10 μg/ml. For the statistical data, resonant frequencies of 5 different resonators were measured for each concentrations and ions.

References

1. S.-p. Han, H. T. Maune, R. D. Barish, M. Bockrath and W. A. Goddard, *Nano Letters*, 2012, **12**, 1129-1135.
2. J. Kim, J. Cho, P. M. Seidler, N. E. Kurland and V. K. Yadavalli, *Langmuir*, 2010, **26**, 2599-2608.
3. E. J. Petersen, X. Tu, M. Dizdaroglu, M. Zheng and B. C. Nelson, *Small*, 2013, **9**, 205-208.
4. T. Premkumar, R. Mezzenga and K. E. Geckeler, *Small*, 2012, **8**, 1299-1313.
5. M. Zheng, A. Jagota, E. D. Semke, B. A. Diner, R. S. McLean, S. R. Lustig, R. E. Richardson and N. G. Tassi, *Nat Mater*, 2003, **2**, 338-342.
6. M. Dresselhaus, G. Dresselhaus and P. Eklund, *Academic Press*, 1996, **5**, 29.
7. X. Li, K. Blinn, Y. Fang, M. Liu, M. A. Mahmoud, S. Cheng, L. A. Bottomley, M. El-Sayed and M. Liu, *Physical Chemistry Chemical Physics*, 2012, **14**, 5919-5923.
8. P. A. Temple and C. E. Hathaway, *Physical Review B*, 1973, **7**, 3685-3697.