Supplementary Material (ESI) for Lab on a Chip

Biosensor *System-on-a-chip* including CMOS-based Signal Processors and 64 Carbon Nanotube-based Sensors for the Detection of a Neurotransmitter

Supplementary Information

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Supplementary Methods

1. Fabrication of Signal Processing Chip

The signal processing chip was designed and fabricated by using 0.18 μm CMOS foundry service (Dongbu-Anam, Korea). The chip was comprised three basic layers: 1) CMOS TR layer, 2) interconnection layers, and 3) pad layers. The CMOS TR layers consisted of all active devices such as transistors (TRs), while each device was connected by metal wires of interconnection layer. The pad layer on top of the chip was comprised of source and drain electrode pads (made of Al) which was obtained by opening the final passivation layer (made of SiO_2 and Si_3N_4). The ADC circuits were located underneath each sensor junction, and they were connected to sensors by the vertical interconnections.

2. Fabrication of Nanotube Circuits

Photoresist (AZ5214) patterns were formed on thermal silicon oxide substrates (Wafermarket, Korea) via photolithography. The sample was dipped into 1:500 (v/v) octadecyltrichlorosilane (Aldrich, USA) solution in anhydrous hexane (Sigma, USA) solution for 2 min, then extensively rinsed with acetone, methanol and ethanol. Then, to assemble CNTs on the polar silicon oxide regions, the substrate was immersed in 0.05 mg/ml carbon nanotube (Carbon Nanotechnologies, USA) suspension in *o*-dichlorobenzene (J. T. Baker, USA) for ~1 min, followed by extensive rinsing with *o*-dichlorobenzene and N_2 drying. To define the electrodes, another photoresist pattern was formed via photolithography, and 10 nm Pd (or Ti) and 20 nm Au were deposited with Multira 1800 thermal evaporator (ODiS, Korea) followed by lift-off process and cleaning with acetone, methanol and ethanol.

3. Glutamate Oxidase Immobilization

The electrode-defined sample was immersed in 1:500 (v/v) 3-aminopropyltriethoxysilane (APTES, Sigma, USA) solution in ethanol (J. T. Baker, USA) solution for 5 min, followed by ethanol rinsing and N_2 drying. This process terminates the substrate region between the adsorbed CNTs with amine group. Afterwards, the sample was treated with 1.25% (v/v) aqueous solution of glutaraldehyde (Junsei, Japan) for ~1hr, followed by rinsing with de-ionized water and N_2 drying.

Then, 20 µl of ~1 mg/ml glutamate oxidase (Yamasa, Japan) in 10 mM pH 7.4 Phosphate Buffer Saline (PBS, Sigma, USA) was dropped over the aldehyde-terminated CNT junction. The humidity was controlled to keep the enzyme solution drop from drying away. The sample was kept in 4□ overnight (~12 hrs). Before performing sensing experiments, the sample was extensively rinsed with PBS to remove residual enzymes. The same process was applied for the immobilization of glucose oxidase.

3. PNA Immobilization

The electrode-defined sample was immersed in 1:500 (v/v) 3-mercaptoprophylmethoxysilane (MPTMS, Aldrich, USA) solution in ethanol (J. T. Baker) for 45 min, followed by ethanol rinsing and N_2 drying. This process terminates the substrate region between the adsorbed CNTs with thiol group. Then, ~20 µl of 7 µM acrydite-functionalized PNA (5'-Acrydite- CCTAATACCAAT-3', Mbiotech, Korea) in deionized water was dropped over the thiol-terminated CNT junction. The humidity was controlled to keep the PNA solution drop from drying away. The sample was kept in 4□ overnight (~12 hrs). Before performing sensing experiments, the sample was extensively rinsed with deionized water to remove residual PNA strands.

4. Activity Assessment of Immobilized Enzymes

The immobilized enzymes glutamate and glucose oxidase were shown to retain their activity by detecting the hydrogen peroxide released by the enzymatic reaction of the immobilized glutamate and glucose oxidase with their respective target molecules, L-glutamate and β-D-glucose, using spectrophotometric analysis at λ_{max} of 432 nm. The reduced *o*-dianisidine is oxidized by the added enzyme hydrogen peroxidase. Enzyme-immobilized samples were immersed in 1 ml of 0.1 mM solutions of respective target molecules, L-glutamate and β-D-glucose. Then, 2 µl of 1 mg/ml hydrogen peroxidase and 200 µl of 0.5 mM *o*-dianisidine was added. After ~12 hrs, the solution acquired a typical brown color visible with the bare eyes.

5. Sensor Experiments with Uniform Array of swCNT-based Transducers

For sensing experiments, the conductance change of the CNT junction was monitored using a Keithley 4200 semiconductor characterization system as the target molecules were injected. A constant source-drain bias in the range of 10 mV to 100 mV was applied. A Pt pseudo-electrode was immersed in the buffer and kept at constant gate voltage of 0 to -0.5 V.

6. L-glutamate Sensing

For sensor experiments, 40 µl of pH 7.4 PBS was dropped on top of the CNT junction and then 10 µl of target substrate was injected. Keithley 4200 semiconductor characterization system was used to monitor the conductance change of the transducer. A Pt pseudo-reference electrode used for liquid gating, using Vg = -0.5 V. The gate current was monitored to avoid electrochemical reactions. Condition was kept so that the current through reference electrode is kept to minimum. L-glutamate was tested in the concentration range of 10 nM to 100 mM. The limit of detection is around 100 nM.

7. Monosodium Glutamate (MSG) Sensing

For sensor experiments, 40 µl of pH 7.4 PBS was dropped on top of the CNT junction and then 10 µl of target substrate was injected. Keithley 4200 semiconductor characterization system was used to monitor the conductance change of the transducer. A Pt pseudo-reference electrode used for liquid gating, using Vg = -0.5 V. The gate current was monitored to avoid electrochemical reactions. Condition was kept so that the current through reference electrode is kept to minimum. A concentration of 1 mM of MSG in the order used in foods was chosen for the experiment. The same sensing method was applied for glucose sensing.

8. DNA Sensing

For sensor experiments, 40 µl of deionized water was dropped on top of the CNT junction and then 10 µl of target substrate was injected. Keithley 4200 semiconductor characterization system was used to monitor the conductance change of the transducer. Non-complementary (5’-GCC ACC GCC GAA-3’, Mbiotech, Korea) and complementary (5’-ATT GTT ATT AGG-3’, Mbiotech, Korea) DNA strands were

injected into PNA functionalized CNT circuit with 22 nM, 13 nM concentration respectively.

Supplementary Figures

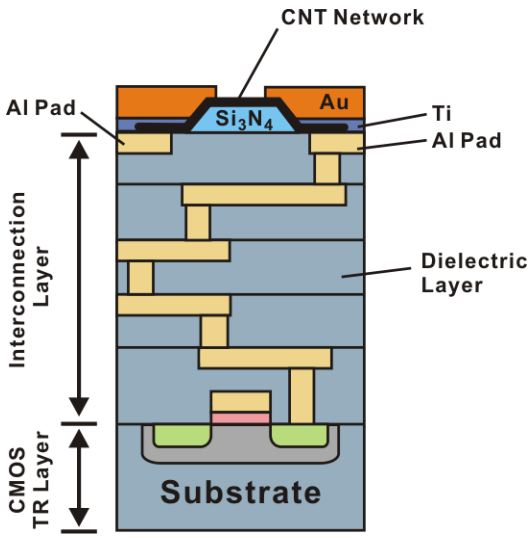

Fig. S1 The section diagram of the CNT-CMOS integrated circuit. CMOS transistors are fabricated on the silicon substrate on which the CNTs are assembled using linker-free assembly method. The signal processing chip was designed and fabricated by using 0.18 μm CMOS foundry service (Dongbu-Anam, Korea).

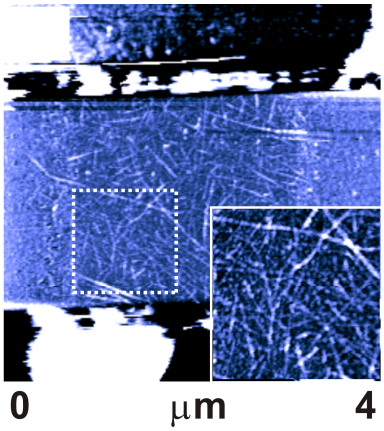

Fig. S2 Atomic force microscope (AFM) topography image of a CNT junction with immobilized glutamate oxidase; (inset) the magnified AFM topography image of the CNT network with immobilized enzyme.

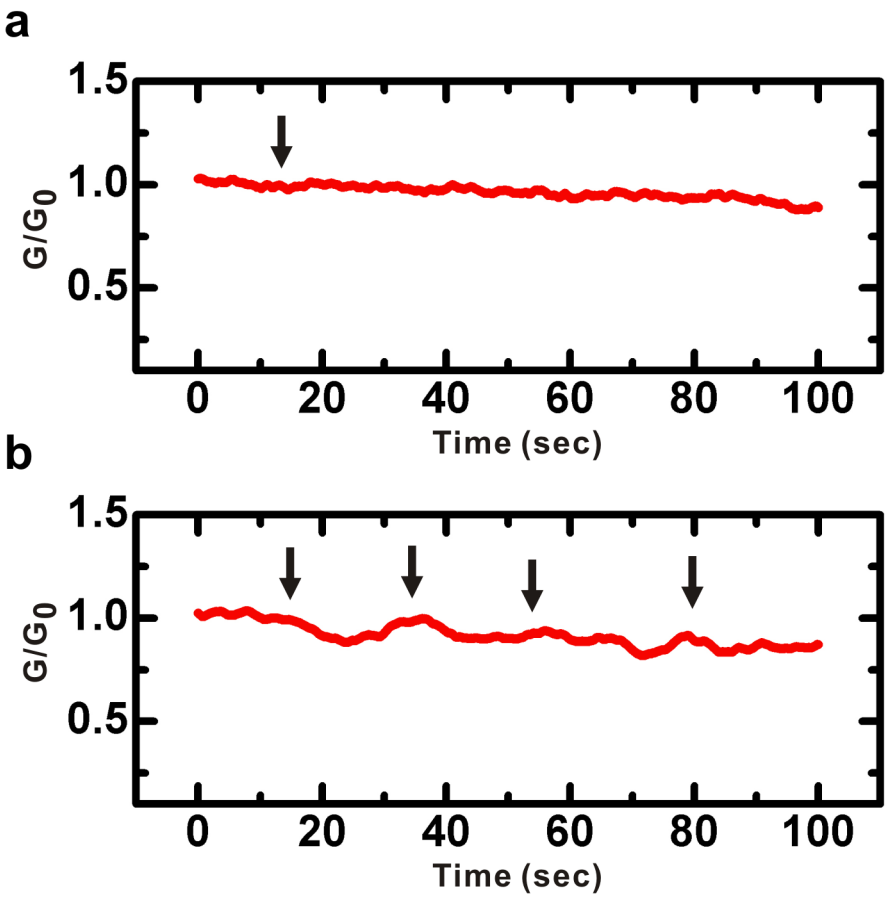

Fig. S3 Control experiments performed to observe the effects of the individual enzymatic reaction of L-glutamate and glutamate oxidase. (a) 1.47 mM hydrogen peroxide injection. (b) 1.8 μM α-ketoglutarate. The arrows show the instant of target molecule injection.

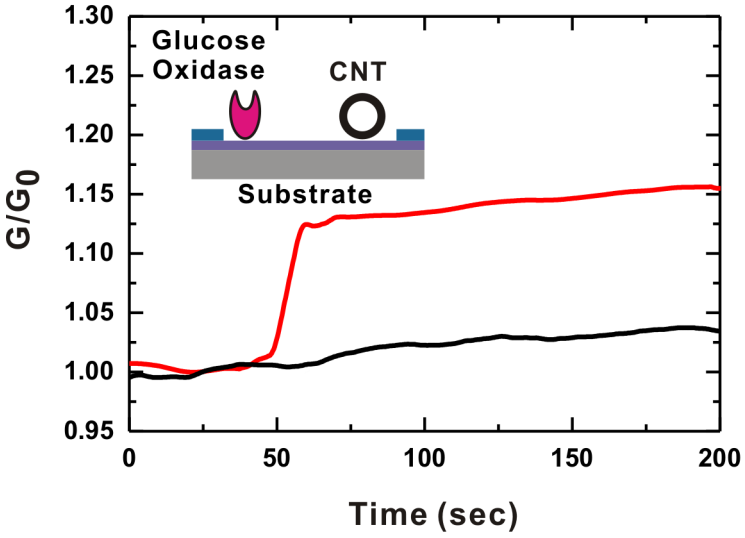

Fig. S4 CNT-based sensors prepared via *substrate functionalization*. 1 μM glucose was detected with (red) and without (black) glucose oxidase immobilized.

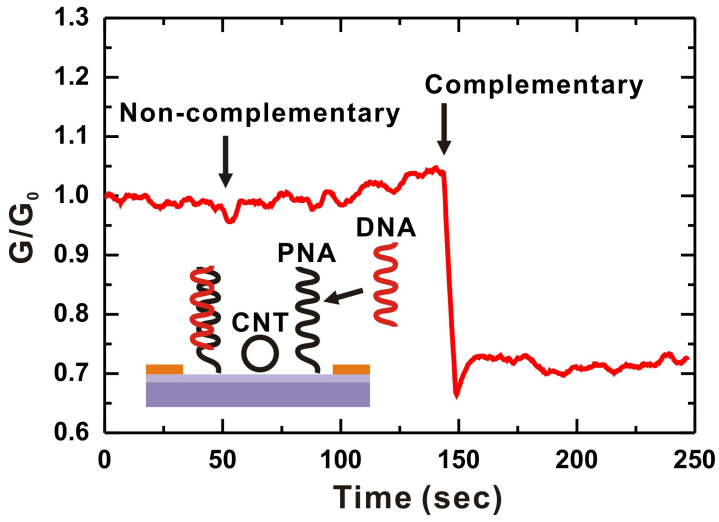

Fig. S5 Detection of 12-bp DNA strand (5'-ATTGGTATTAGG-3'). The junction showed no response to 22 nM non-complementary sequence DNA (5'-GCC ACC GCC GAA-3'), while the injection of 13 nM complementary sequence DNA reduced the conductance of swCNT junctions by ~30%.