Electronic Supplementary Information (ESI) for Lab on a Chip

"Bioelectronic Super-taster" Device based on Taste Receptor-Carbon Nanotube Hybrid Structures

Supplementary Information

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SDS-PAGE and Western Blotting analysis

Samples were mixed with SDS sample buffer (10% sodium dodecyl sulfate, 10% β-mercaptonethanol, 0.3 M Tris-HCl (pH 6.8), 0.05% bromophenol blue, 50% glycerol) and incubated at 50 ºC for 2 hr. The same amount of samples were loaded onto 10% PAGE gels (Laemmli) and electrophoresed at 80 V. Proteins in the gels were visualized with 0.02% Coomassie-blue staining solution. For western blotting, proteins in the gels were transferred to nitrocellulose membranes. The membranes were incubated for 2 hr with 5% skim milk in PBS-T (PBS containing 0.1% Tween-20) for blocking. The blocked membranes were incubated with anti-GST antibody (from mouse, 1:2,000 dilution with 1% skim milk in PBS-T: Santa Cruz, CA, USA) for 1 hr at room temperature, and then washed with PBS-T (10 min, 5 times). Primary anti-body treated membranes were incubated with HRP-conjugated anti-mouse antibody (1:2,500 dilution with 5% skim milk in PBS-T: GE Healthcare) and washed with PBS-T (10 min, 5 times). Western blotting was performed using an ECL kit (GE Healthcare, WI, USA).

Fabrication of swCNT-FETs

The photoresist (AZ5214) was first spin coated at a speed of 500 rpm for 10 s, 4000 rpm for 45 s and 500 rpm for 5 s on the SiO_2 (1000Å) substrate, and it was patterned via pho tolithography using MA6 (Karl Suss, Germany). The metal (30 nm Au on 10 nm Ti) was deposited using thermal evaporator (Multira 1800, Odis, Korea). After that, an align key was patterned by lift-off process.

Next, the photoresist was patterned on the substrate again for surface molecular patterning. Then, the patterned substrate was dipped in the octadecyltrichlorosilane (OTS) solution (OTS:anhydrous hexane=1:500 (v/v), Sigma, USA) for 10 min inside the humidity chamber maintained a temperature of 23 ºC and a humidity of 80%. Then, the substrate was immediately rinsed with clean anhydrous hexane to prevent the OTS aggregation. After that, the photoresist was removed by acetone, and ethanol followed by heating in a convection oven at a temperature of 50 ºC for 10 min to enhance OTS f unctionalization on the oxide layer.

For swCNT (CNR, USA) assembly, swCNT suspension (0.05 mg ml^{-1} in 1, 2-dichloro benzene, JUSEI, Japan) was prepared. When the sample was dipped in swCNT suspensio ns for ~5 s, swCNTs were selectively adsorbed and aligned on the bare SiO_2 surface regions. Then, the substrate was thoroughly rinsed with 1, 2-dichlorobenzene and de-ionized (DI) water.

Finally, source and drain electrodes (30 nm Au on 10 nm Pd) were patterned by conventional lift-off process. It should be noted that the entire processes, including mol ecular patterning, could be performed using conventional microfabrication facilities.

Measurement Procedure

We carried out an extensive control experiment to measure the effect of tastants on a swCNT-FET functionalized with hTAS2R38. First, we prepared each tastant diluted in phosphate buffered saline (PBS) solution from 10 fM to 10 mM concentration for the preparation of the tastant stock solution. Then, human taste receptor functionalized swCNT-FET was placed on the plate in shielding box. Next, electrodes were connected with Keithly 4200 semiconductor parameter analyzer to introduce voltage and measure current. Then, a 9 μL droplet of PBS was placed on the junction of hTAS2R38-functionalized swCNT-FET. The

source–drain current was then monitored after the introduction of a specific tastant solution. A 100 mV bias voltage was maintained as a source-drain voltage at all times during electrical measurement. After that, we first injected 1 L droplet of 10 fM tastant solution to make final concentration 1 fM. For other concentrations, we also added appropriate amount of the tastant stock solution to PBS solution on swCNT-FET. As a result, we could get a real time sensing data of tastant from 1 fM to 1 mM increased by 10 times.

Supplementary Figures

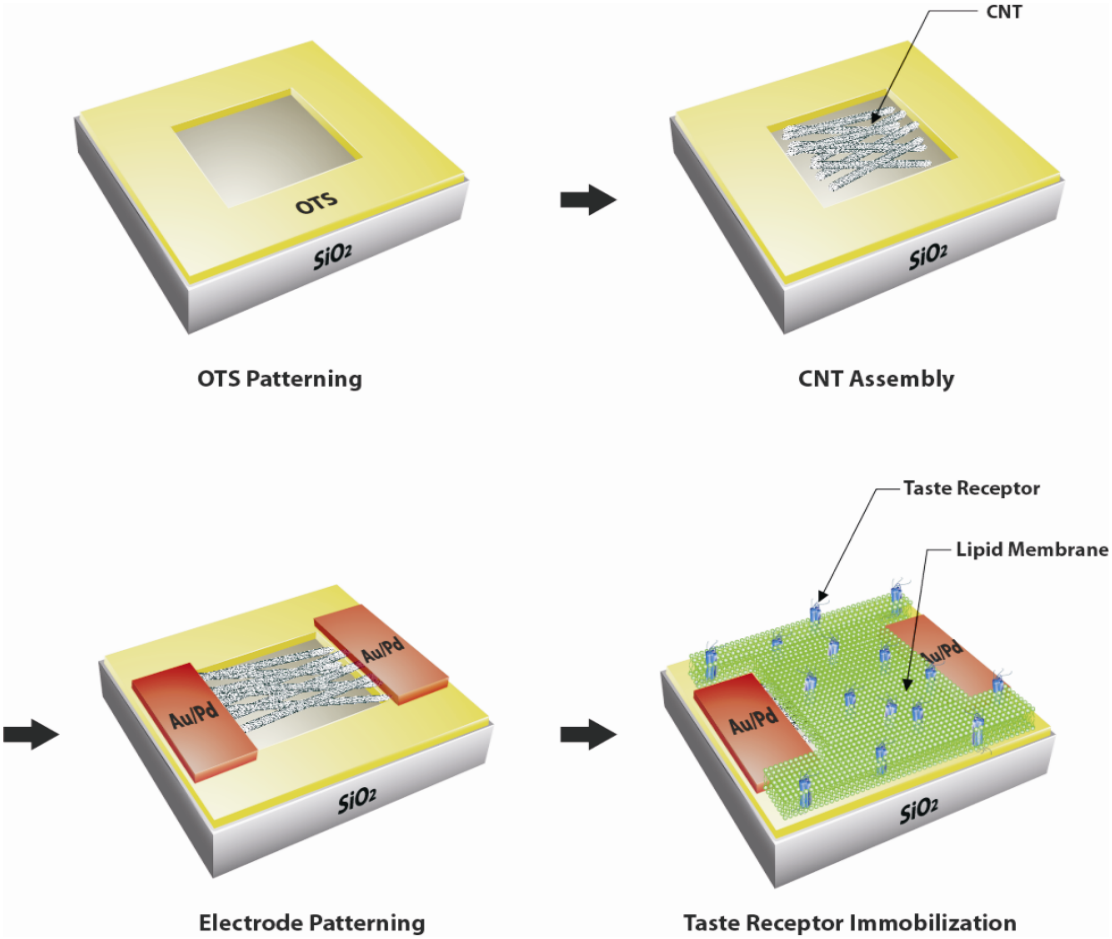

Fig. S1. Schematic diagram showing the fabrication method for a bioelectronic taste sensor based on human taste receptors.

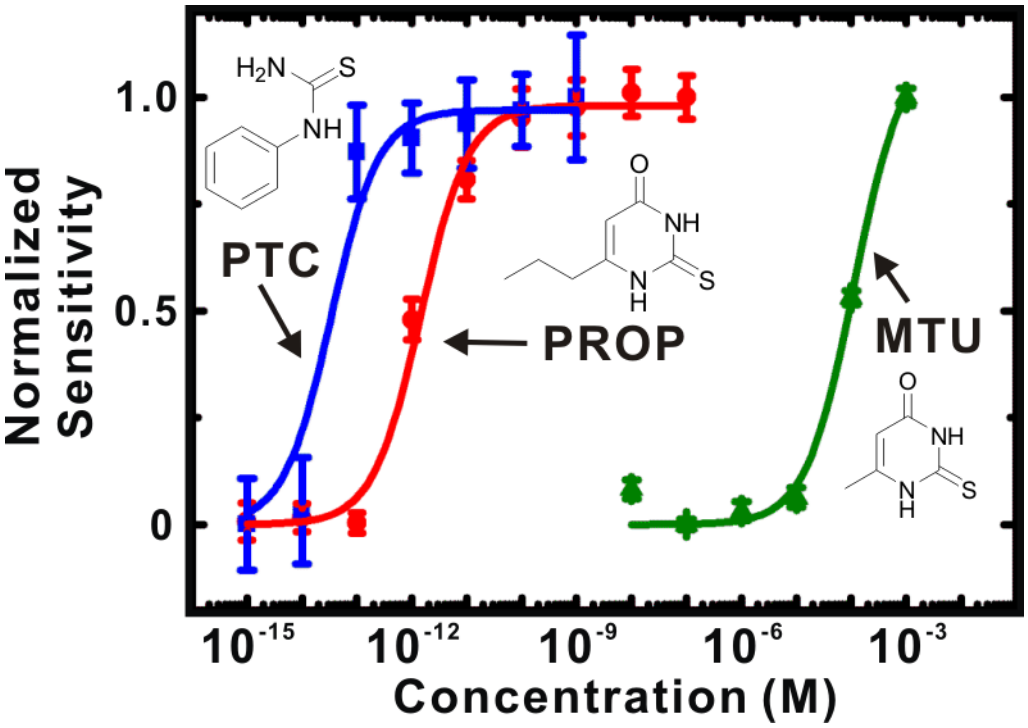

Fig. S2. Measured data (dots) and fitting curves (solid lines) of BST device responses to PTC (target bitter tastant), PROP (target bitter tastant), and MTU (non-target bitter tastant). Note that we fitted the data using the equation including the *ligand depletion effect*.

The ligand depletion may occur when the number of receptor molecules is much larger than that of tastant molecules. In this case, the concentration of tastant solution after sensor response may be much lower than that of original tastant solution. Considering the ligand depletion (Ref. 35 and 36), the surface density C_s of tastant molecules bound to tastant receptors on BST device surface can be written as

$$C_s = (C_R + C_T + K) - \sqrt{(C_R + C_T + K)^2 - 4 \cdot C_R \cdot C_T} \qquad \text{Equation (S1)}$$

where C_R and C_T represent the concentration of recepotrs and tastants, respectivley. Since the sensor response $|\Delta G/G_0|$ can be written as $|\Delta G/G_0| \sim kC_s$ as explained in the text part, we can write

$$|\Delta G / G_0| = k\left((C_R + C_T + K) - \sqrt{(C_R + C_T + K)^2 - 4 \cdot C_R \cdot C_T}\right) \qquad \text{Equation (S2)}$$

Equation (S2) is the equation describing BST responses including the ligand depletion effect, and we utilized it to fit the measured response data. The estimated K values for PTC, PROP, and MTU by this fitting are 3.0 x 10^{13} M^{-1}, 6.9 x 10^{11} M^{-1}, and 9.0 x 10^{4} M^{-1}, respectively. Note they are different only by ~10% or less from the estimated K values using the equation (2) which does not consider the ligand depletion effect. Although it is still uncertain about the degree of ligand depletion in our experiments, these results imply that, at least, the first digit of the estimated K values is reliable and uncertainty occurred from the second digit of the measured K values.

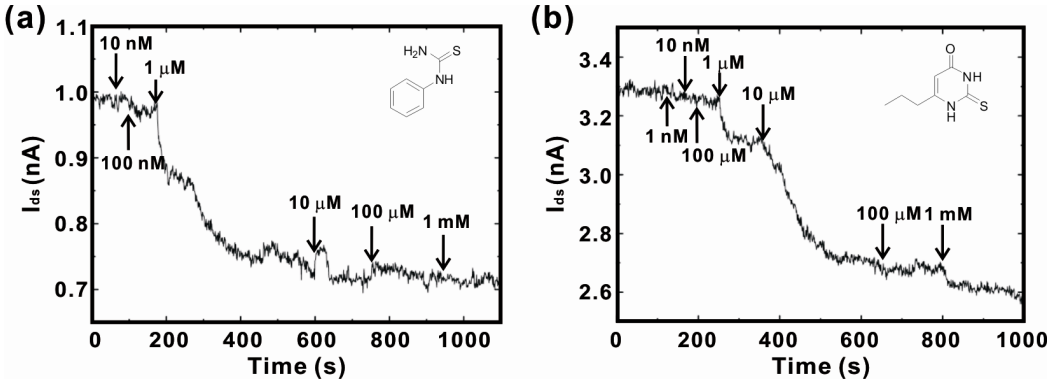

Fig. S3. Non-specific response of bare swCNT-FETs after the introduction of target a) PTC and b) PROP at various concentrations. Arrows indicate the instances of tastant injections.

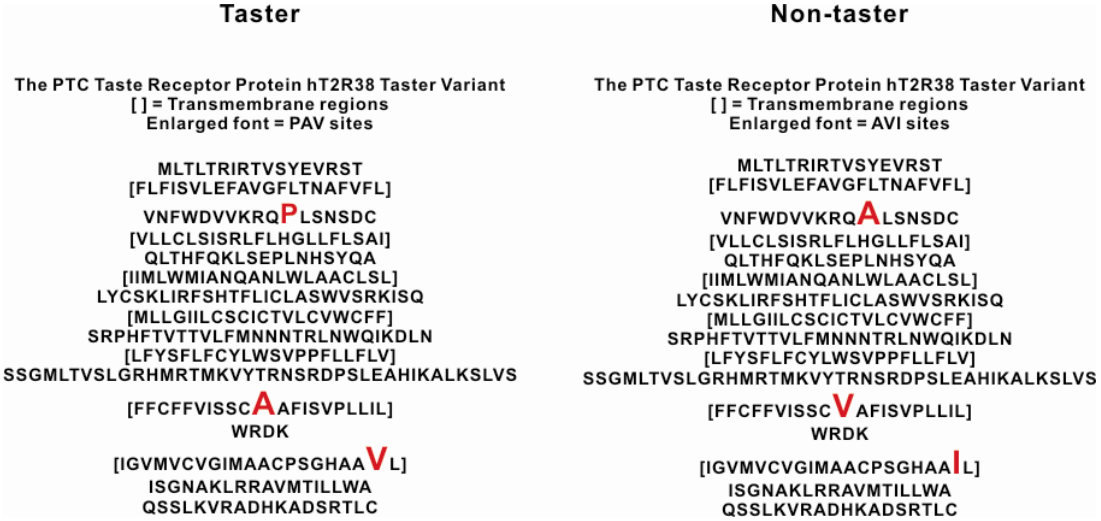

Table S1. Polymorphisms within the hTAS2R38 (PTC) gene have been reported by U.-k. Kim *et al.* (*Science* **299**, 1221 (2003)). Sequences of syllables can be noted within and without [ ]. Lines without [ ] exhibit sequences of amino acid in an intracellular or extracellular loop while those within [ ] represent sequences of amino acid in the transmembrane region. The amino acid sequences of the tasters and non-tasters are identical except for the three depicted in a red enlarged font.

Tastant	Detection Limit
PTC	100 fM
PROP	1 pM
MTU	100 μM
L-Glutamic Acid	No response
Sucrose	No response

Table S2. Detection limits of BST devices based on PAV- type hTAS2R38 for different tastants.

FET	Detection Limit	
	PTC	PROP
swCNT-FETs	1 μM	1 μM
BST devices based on PAV-type hTAS2R38	100 fM	1 pM
BST devices based on AVI-type hTAS2R38	100 nM	100 nM

Table S3. Detection limits of swCNT-FETs and BST devices for PTC and PROP.