

# Bioelectronic Nose Using Odorant Binding Protein-Derived Peptide and Carbon Nanotube Field-Effect Transistor for the Assessment of *Salmonella* Contamination in Food

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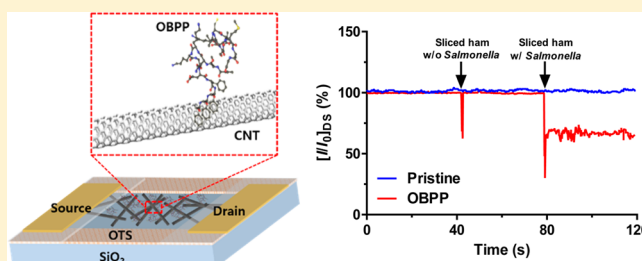
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## Supporting Information

**ABSTRACT:** *Salmonella* infection is one of the major causes of food borne illnesses including fever, abdominal pain, diarrhea, and nausea. Thus, early detection of *Salmonella* contamination is important for our healthy life. Conventional detection methods for the food contamination have limitations in sensitivity and rapidity; thus, the early detection has been difficult. Herein, we developed a bioelectronic nose using a carbon nanotube (CNT) field-effect transistor (FET) functionalized with *Drosophila* odorant binding protein (OBP)-derived peptide for easy and rapid detection of *Salmonella* contamination in ham. 3-Methyl-1-butanol is known as a specific volatile organic compound, generated from the ham contaminated with *Salmonella*. We designed and synthesized the peptide based on the sequence of the *Drosophila* OBP, LUSH, which specifically binds to alcohols. The C-terminus of the synthetic peptide was modified with three phenylalanine residues and directly immobilized onto CNT channels using the  $\pi$ - $\pi$  interaction. The p-type properties of FET were clearly maintained after the functionalization using the peptide. The biosensor detected 1 fM of 3-methyl-1-butanol with high selectivity and successfully assessed *Salmonella* contamination in ham. These results indicate that the bioelectronic nose can be used for the rapid detection of *Salmonella* contamination in food.



*Salmonella* is a bacterium that causes food poisoning by the consumption of contaminated food.<sup>1</sup> Symptoms include fever, malaise, abdominal pain, headache, myalgia, nausea, anorexia, and constipation, which threatens one's health.<sup>2</sup> For the effective detection of *Salmonella* contamination, various methods such as enzyme immunoassay,<sup>3</sup> antibody capture,<sup>4</sup> DNA probes,<sup>5</sup> and PCR assay<sup>6</sup> have been developed. However, conventional methods have low sensitivity, thus limiting the early detection of pathogens. Gas chromatography–mass spectrometry (GC/MS) has also been used to analyze volatile organic compounds (VOCs) of food contaminated with *Salmonella*, because VOCs such as 3-methyl-1-butanol and 1-hexanol were specifically detected in the headspace of *Salmonella*-contaminated ham.<sup>7</sup> However, GC/MS still has disadvantages of long analytical time and complex operations with high cost.

Recently, bioelectronic sensors using olfactory receptors and nanomaterials were reported for the rapid and simple detection of chemicals. The biosensors mimic the natural smell sensing

mechanism that selectively recognizes the odorants. The integration of biomolecules and nanomaterials, such as carbon nanotubes,<sup>8–15</sup> conducting polymer,<sup>16,17</sup> and graphene,<sup>18,19</sup> could allow us to detect the minute change in the bioelectrical potentials. The biosensors, named as the bioelectronic nose, could be applied to disease diagnosis,<sup>12</sup> environmental monitoring,<sup>15</sup> and food safety control.<sup>10,11,13</sup>

Herein, we present another bioelectronic nose, which can be used for the detection of *Salmonella* contamination in ham, using *Drosophila* odorant binding protein (OBP)-derived peptide and carbon nanotube field-effect transistor (CNT-FET). When odor molecules stimulate the olfactory system, OBP, which is a soluble protein in olfactory mucus, binds with the odorant and transfers it to olfactory receptors.<sup>20</sup> Combinatorial binding patterns of olfactory receptors and

**Received:** August 22, 2016

**Accepted:** November 14, 2016

**Published:** November 14, 2016

odorants send electrical signals to higher regions of the brain, and finally, smell is perceived.<sup>21</sup> LUSH is an OBP of *Drosophila* that specifically binds to alcohols.<sup>22</sup> The structure of LUSH was defined, and the specific binding site for alcohol was previously reported.<sup>23</sup> OBPs have been used for the development of biosensors which were combined with surface plasmon resonance,<sup>24</sup> quartz crystal microbalance,<sup>25</sup> surface acoustic wave,<sup>26–28</sup> cantilever,<sup>29</sup> electrochemical impedance spectroscopy,<sup>30,31</sup> and FET.<sup>32</sup> In this study, the peptide, which is derived from LUSH, was synthesized by the modification of the C-terminus with three additional phenylalanine (Phe) residues, and it includes specific binding site residues of LUSH. The synthesized peptide was directly immobilized onto CNT surfaces via the  $\pi$ - $\pi$  stacking interactions between Phe residues and CNTs.<sup>33,34</sup> The peptide-based bioelectronic nose that is inspired by the insect olfactory systems could detect 3-methyl-1-butanol with high specificity and selectivity. Furthermore, the bioelectronic nose could be used for the rapid assessment of *Salmonella* contamination by simply diluting ham samples.

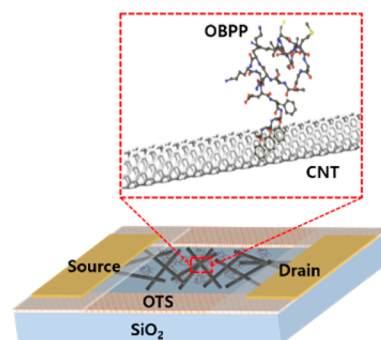
## EXPERIMENTAL SECTION

The CNT-FET was fabricated via the photolithography method as previously reported.<sup>35</sup> Briefly, AZ5214 photoresist (PR) was used to fabricate a pattern on a SiO<sub>2</sub> wafer. The wafer was dipped into octacyltrichlorosilane (OTS) solution (OTS/hexane = 1:500) for 7 min. The wafer was rinsed with hexane, acetone, and ethanol to remove the PR patterns. The OTS-patterned substrate was incubated in swCNT solution (0.02 mg/mL in 1,2-dichlorobenzene) for 30 s, and CNTs were specifically adsorbed to the substrate. Afterward, Pd/Au (10/30 nm) electrodes were fabricated and passivated using PR to protect them from contacting the solution. The *Drosophila* OBP-derived peptide (TKCVSLMAGTVNKKGEFFFF) was synthesized with purity higher than 90% by Pepton (Korea). The peptide was diluted at 1  $\mu$ g/mL in distilled water and stored at -20 °C. For the immobilization of the peptide, 1.5  $\mu$ L of peptide solution was placed on the CNT channel and incubated for 4 h at room temperature. After the immobilization process, unbound peptides were washed out 2–3 times with distilled water. An atomic force microscopy (AFM) system (MFP-3D, Asylum Research, USA) was used to analyze the immobilization of the peptide. The pristine CNT channel was imaged at a scan rate of 0.2 Hz. After the peptide immobilization, the CNT channel was imaged again at 0.05 Hz.

All the odorants were sequentially diluted to 1:10 using distilled water and stored at 4 °C until used. Sliced ham (Korea) was purchased from a local market and stored at 4 °C. To prepare a *Salmonella*-contaminated food sample,  $4 \times 10^7$  CFU mL<sup>-1</sup> of *Salmonella typhimurium* LT2 was spiked on sliced ham samples, and then, the ham samples were stored at 25 °C for 4 days.<sup>36</sup> After the storage, 1 mL of distilled water was added to 1 mg of the food samples and diluted to 1:100 before use in the experiments.<sup>11</sup> For measuring the current signal, 20  $\mu$ L of distilled water was added on the CNT channel, and 2  $\mu$ L of odorant or food samples was injected into the CNT channels using micropipettes. A bias voltage of 0.1 V was applied to the source and drain electrodes, and the gate voltage was grounded. Current signals were monitored using a 2326A source meter (Keithley, USA) and a MST 800 probe station (MS TECH, Korea). The bioelectronic sensors were used for only one measurement.

## RESULTS AND DISCUSSION

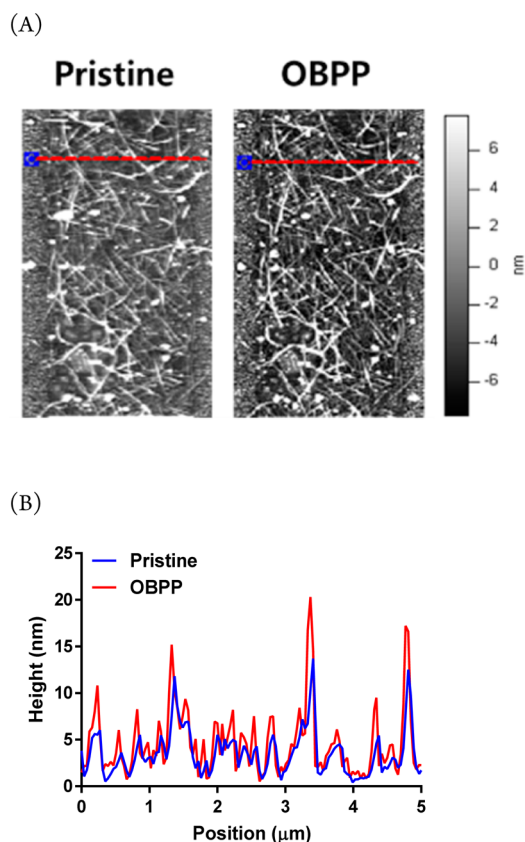
For the detection of 3-methyl-1-butanol, which is an indicator of *Salmonella* contamination, *Drosophila* OBP-derived peptide (OBPP) has to be synthesized on the basis of the sequence and structure in the *Drosophila* OBP, LUSH. The three-dimensional structure of the LUSH was previously identified.<sup>23</sup> Thr57, Ser52, and Thr48 form a network of hydrogen bonds with alcohols providing a structural motif to increase binding affinity. Another research group also reported that Thr57 and Phe64 are essential amino acid residues that bind with ligands.<sup>25</sup> The sequence of the synthesized peptide was TKCVSLMAGT-VNKKGEFFFF. The three Phe residues were added to the C-terminus of the peptide for the immobilization onto CNTs via the  $\pi$ - $\pi$  stacking interaction. Only the original part of the peptide is free, while the three Phe residues are immobilized on CNT. Therefore, it is considered that the Phe addition does not change the three-dimensional structure and the binding affinity of the peptide. Moreover, the peptide isoelectric point calculated with Compute pI/Mw<sup>37</sup> was not changed and was maintained at 9.19 even after the addition of three Phe residues, since Phe is an uncharged residue. The peptide was directly immobilized on the CNT channel as a self-assembled monolayer, so that the field-effect can occur when the structure of the peptide is changed with the binding of 3-methyl-1-butanol (Figure 1). The AFM images of the CNT channel were



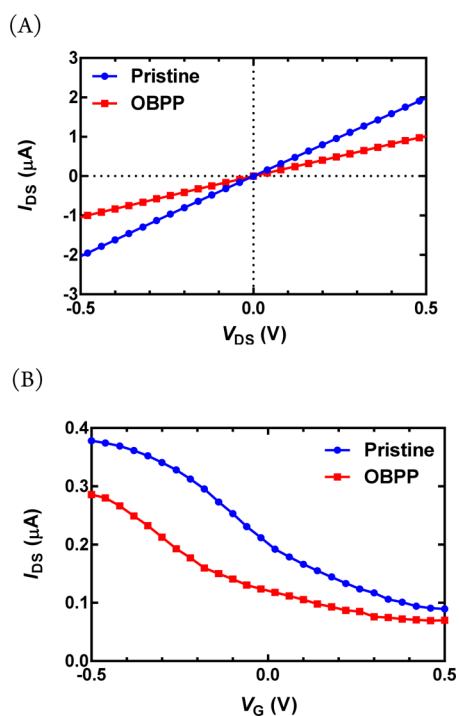
**Figure 1.** Schematic diagram of the bioelectronic nose using carbon nanotube field-effect transistor functionalized with odorant binding protein-derived peptide. The peptides were directly immobilized via  $\pi$ - $\pi$  interactions between Phe residues and CNTs.

measured before and after the immobilization of the peptide (Figure 2A). The height of the CNTs was scanned along the 5  $\mu$ m region, and the average value of the height difference between two cases was calculated. The average height along the 5  $\mu$ m region increased by  $2.05 \pm 2.01$  nm (*p*-value 0.0079) (Figure 2B) after the immobilization process. Because the peptide length calculated with Hydropathy plot<sup>38</sup> was 2.19 nm, it is considered that the peptides were appropriately immobilized on the CNT channel.

To analyze the electrical characteristic of the biosensor, the drain-source current–voltage ( $I_{DS}$ - $V_{DS}$ ) curves of the CNT channel were measured before and after the immobilization of the peptide (Figure 3A). The resistance of the CNT channel was increased from  $2.11 \times 10^6$  to  $3.98 \times 10^6$   $\Omega$ , and the linearity of the  $I_{DS}$ - $V_{DS}$  curve was maintained. When we increased the liquid gate voltage in distilled water, the p-type FET characteristic was observed even after the immobilization process showing the decrease in the current ( $I_{DS}$ ) with increasing gate voltage ( $V_G$ ) (Figure 3B). These results indicate that the peptide was successfully immobilized onto the CNT



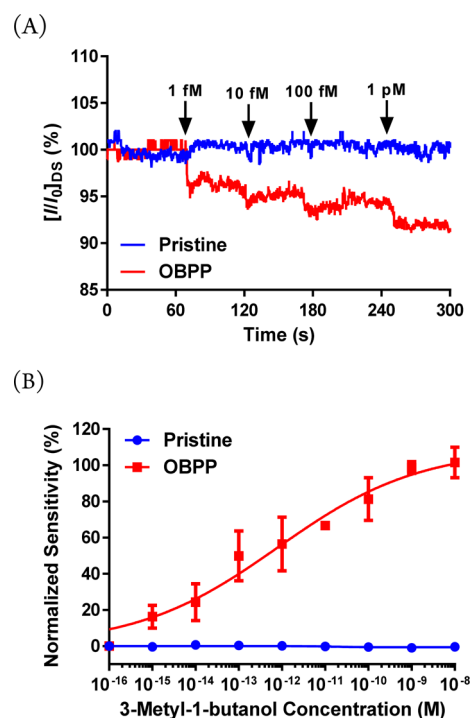
**Figure 2.** Fabrication of the peptide-based CNT-FET. (A) AFM images and (B) height profiles of CNT channels before and after the immobilization of OBPP.



**Figure 3.** Electrical properties of the peptide-based CNT-FET. (A) Current–voltage curves and (B) liquid gate profiles of the CNT channels before and after the immobilization of OBPP.

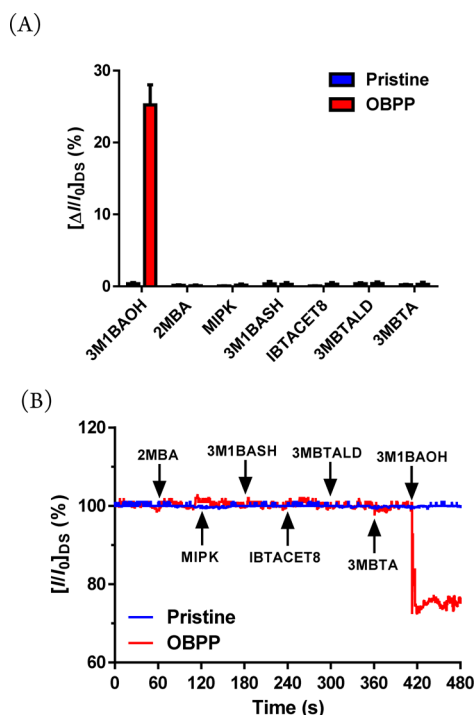
channel and the biosensor could be used for the detection of odorants.

The sensitivity and selectivity of the biosensor was demonstrated using 3-methyl-1-butanol, a standard odor that indicates *Salmonella* contamination in ham. Twenty  $\mu\text{L}$  of distilled water was placed on the CNT channel of the biosensor, and different concentrations of the odor solution were sequentially injected. Figure 4A shows the real-time



**Figure 4.** (A) Real-time conductance changes to various concentrations of 3-methyl-1-butanol using the pristine and OBPP-functionalized CNT-FET. (B) Dose–response curves of the bioelectronic nose to 3-methyl-1-butanol. The signals were normalized by the maximum response of the biosensor ( $10^{-8}$  M). Error bars, s.e.m. of three replicates.

conductance change with various concentrations of 3-methyl-1-butanol. The signal intensity is the difference between the final current value and the initial baseline, which is the initial current value at 0 M of 3-methyl-1-butanol. Prior to the injection of the distilled water, the diluent did not show significant change (data not shown), and no signal changes were observed at lower concentration than 1 fM. The biosensor exhibited the detection limit of 1 fM, and the signal increased with additional injection of higher concentrations. No significant signals were observed for the pristine CNT. The dose-dependent response of the biosensor was normalized by its maximum value and analyzed using the Langmuir isotherm model as previously reported (Figure 4B).<sup>11</sup> The calculated equilibrium constant,  $K_d$ , was  $5.25 \times 10^{13} \text{ M}^{-1}$ . Previously, we have tested the sensor selectivity with different lengths of carbon chains.<sup>8,16</sup> Herein, we focused on the functional group selectivity of the sensor, since LUSH is the OBP that specifically binds with alcohol, which has a hydroxyl group. Six control compounds, with chemical structures similar to 3-methyl-1-butanol, did not show any response for both the pristine CNT and the OBPP-functionalized CNT (Figure 5A). LUSH is the OBP that specifically binds to alcohol. OBPP is the synthetic peptide



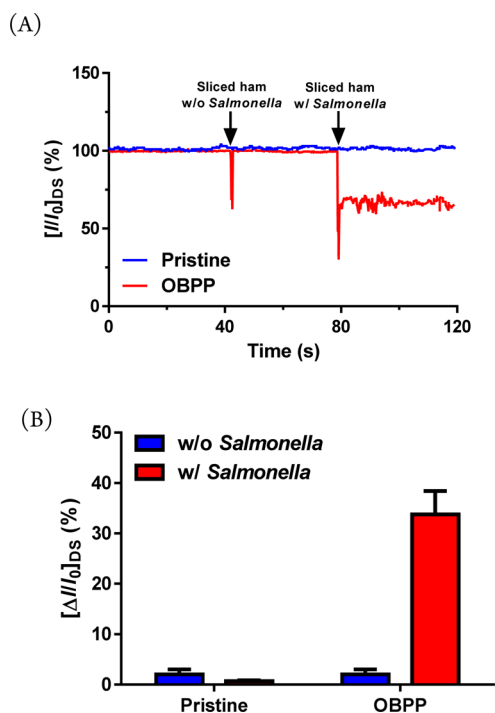
**Figure 5.** (A) Specific recognition of 3-methyl-1-butanol from other molecules in real-time. Five odorants including 3-methyl-1-butanol were applied to the bioelectronic nose in real-time for testing the selectivity of the biosensor, and all odorant concentrations were fixed at 1 nM. (B) Quantitative comparison of conductance changes with the injection of odor molecules. Error bars, s.e.m. of three replicates. The abbreviations used are as follows: 3M1BAOH, 3-methyl-1-butanol; 2MBA, 2-methylbutane; MIPK, methyl isopropyl ketone; 3M1BASH, 3-methyl-1-butanethiol; IBTACET8, isobutyl acetate; 3MBTALD, 3-methylbutanal; 3MBTA, 3-methylbutanoic acid.

derived from the LUSH. The OBPP has the specific alcohol-binding site of the LUSH,<sup>23</sup> so that the bioelectronic nose can selectively distinguish relevant odorants. Quantitative comparison of 3-methyl-1-butanol to the control compounds shows that the biosensor selectively responded to 3-methyl-1-butanol (Figure 5B). These results indicate that the biosensor could sensitively and selectively detect the specific odorant generated from the *Salmonella*-contaminated ham.

When ham is contaminated by *Salmonella*, 3-methyl-1-butanol is generated and this molecule is regarded as an indicator of contamination.<sup>7</sup> To verify that the biosensor can be used for the assessment of food contamination, fresh and *Salmonella*-contaminated ham samples were injected to the biosensor. While the biosensor did not respond to a fresh ham sample, a significant signal was observed with a response time of less than 2 s when a *Salmonella*-contaminated ham sample was injected (Figure 6A). The pristine CNT did not respond to the *Salmonella*-contaminated ham sample as expected. Furthermore, inactivated *Salmonella* did not show any responses to the sensor (Figure S1). Thus, the detection of *Salmonella*-contaminated ham samples was available with the OBPP-functionalized CNT channel (Figure 6B). The simple manipulation and rapid detection of the biosensor allowed for the effective assessment of ham contamination by *Salmonella*.

## CONCLUSION

In this study, an OBPP-based CNT biosensor was developed for the rapid assessment of *Salmonella* contamination in ham.



**Figure 6.** (A) Real-time detection of *Salmonella* contamination in sliced ham. (B) Quantitative comparison of conductance changes with the injection of noncontaminated and contaminated ham samples. Error bars, s.e.m. of three replicates.

LUSH is known as a *Drosophila* odorant binding protein to bind with alcohols. LUSH (*Drosophila* OBP)-derived peptide, containing essential amino acid residues for binding to alcohols, was synthesized with three additional phenylalanine residues at the C-terminus of the peptide and directly immobilized onto CNT channel of the biosensor via  $\pi$ - $\pi$  interactions between Phe residues and CNTs. The peptide-based biosensor sensitively detected 3-methyl-1-butanol at a concentration of 1 fM and selectively distinguished the target odor molecule from other compounds with similar structures. Moreover, the biosensor detected the *Salmonella*-contaminated ham sample without a complex pretreatment process. Taken together, the bioelectronic nose, which relies on the peptide based on the insect OBP, can be used for the detection of *Salmonella* contamination in ham with a rapid and simple process. It is also expected that this approach can be applied to various food samples by using other OBPs or olfactory receptors which specifically bind with different food contaminants.

## ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.analchem.6b03284.

Real-time response of pristine and OBPP-functionalized CNT-FET to inactivated *Salmonella* (Figure S1) (PDF)

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## Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

## Notes

The authors declare no competing financial interest.

## ACKNOWLEDGMENTS

This research was supported by the Public Welfare & Safety Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Science, ICT & Future Planning (2014039771), the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIP) (2014053108, 2013M3A6B2078961), and the Center for Integrated Smart Sensors funded by the Ministry of Science, ICT & Future Planning as Global Frontier Project (CISS-2011-0031870).

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