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Multifunctionalized Cantilever Systems for Electronic Nose Applications

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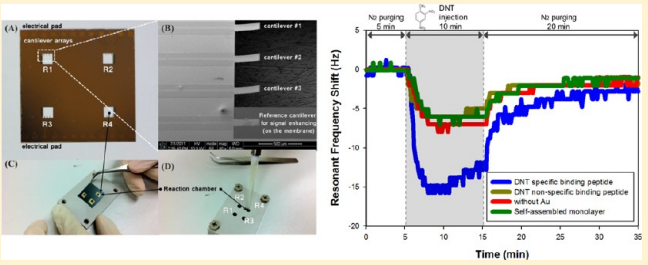
ABSTRACT: Multiple target detection using a cantilever is essential for biosensor, chemical sensor, and electronic nose systems. We report a novel microcantilever array chip that includes four microreaction chambers in a chip, which consequently contains four different functionalized surfaces for multitarget detection. For model tests, we designed microcantilever chips and demonstrated the ability of binding of 2,4-dinitrotoluene (DNT) targets onto four different surfaces. We used peptide receptors that are known to have highly selective binding. By simply using four microreaction chambers, we immobilized DNT specific peptide (HPNFSKYILHQRC; SP), DNT nonspecific peptide (TSMLLMSPKHQAC; NSP), and self-assembled monolayer (SAM) as well as a bare cantilever. After flowing DNT gases through the cantilever chip, we could monitor the four different binding signals simultaneously. The shifts in NSP provided information as a negative control because it contained information of temperature fluctuations and mechanical vibration from gas flow. By utilizing the differential signal of the SP and NSP, we acquired 7.5 Hz in resonant responses that corresponds with 160 part per billion (ppb) DNT concentration, showing the exact binding response by eliminating the inevitable thermal noise, vibration noise, as well as humidity effects on the peptide surface.



Recently, great advances have been achieved in bio/chemical sensors. Compared with traditional bio/chemical sensors, such as fluorescence, surface plasmon resonance (SPR), and electrochemical,[1−4] the fundamental merit of nanomechanical translation using cantilevers for bio and chemical detection is that it can provide a common platform for high-throughput analysis, such as protein, DNA, and cell.[5−11] Moreover, the direct transducing ability of target binding onto a receptor immobilized cantilever has the unique merits of fast response and high sensitivity. Also, advances in micro/nano technology are providing better sensing ability, with high sensitivity and selectivity.[12,13]

Importantly, the selectivity of the sensor is the essential criteria in artificial olfactory systems because it provides the discriminating ability between different substances.[7,14] Also, multiple target detection is the most critical criteria in artificial olfactory systems. Generally, most commercially developed receptors have limited selectivity, so that one needs several receptors and to use the principal component analysis (PCA) method.[15,16] As a receptor, the peptide has received great attention, because of its high selectivity.[17,18] We recently showed advances in selectivity with 2,4-dinitrotoluene (DNT) selective peptide,[7] via direct electric signal from the vibrating frequency change using a piezoelectric cantilever.[5,6] From the previous work, we reported explosive DNT detection with high sensitivity as well as selectivity by using a DNT specific receptor. However, we could not realize a simultaneous multitarget detection with individual functionalized cantilevers; consequently we did not acquire real time electric signals from individual cantilevers. If one can acquire signals from individual cantilevers simultaneously, one can address inevitable problems in mechanical damping and thermal expansion coefficient mismatching, caused from instant environmental changes, such as viscosity, density, humidity, and temperature.

Previous reports commonly used surface functionalizing of the cantilever for multitarget detection, which mainly relies on capillary tube and inkjet printing.[19−21] The capillary method utilized commercial capillary tube, by direct insertion onto cantilevers,[22] while the latter used an inkjet printing method for supplying small quantity of droplets onto cantilever surfaces.[21] While these methods provide a multiple function, the major issue of inkjet printing method is that a droplet could rapidly evaporate because of its small volume as well as its high surface to volume ratio. The capillary method could prevent the evaporation problem by continuous fluid feeding. However, cross-contamination generally occurs by capillary action that could lead to fluid flows to adjacent cantilevers, through the edge of cantilevers. Moreover, mechanical devices can be broken without careful handling.

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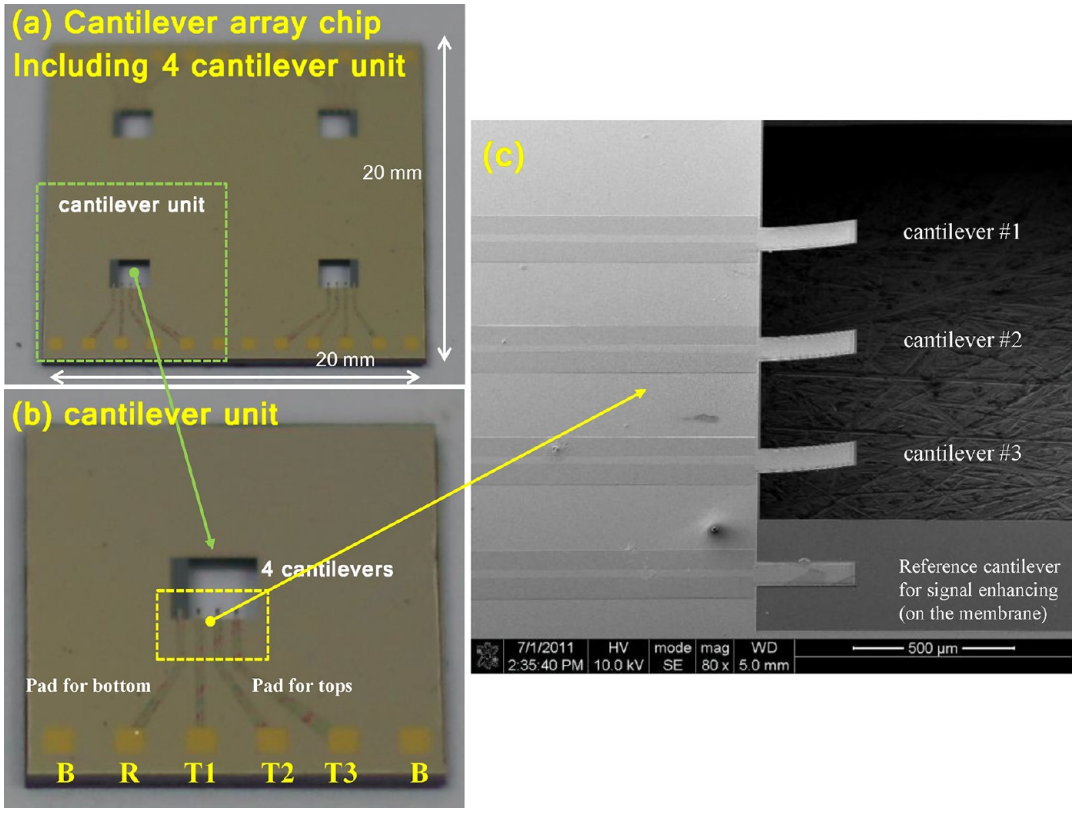

Figure 1. Cantilever array chip with four reaction chambers: optical image showing (a) cantilever including four cantilever units for multiple target detection and (b) one cantilever unit containing cantilever arrays; (c) SEM photograph of one cantilever unit. Note that B and R were bottom Pt electrode (ground) and reference electrode, respectively. T1, T2, and T3 were the top Pt electrodes for the three PZT cantilevers.

To address the problems above as well as provide multifunctionalized surfaces, we introduce a multiple detection technique with four microreaction chambers. As a feasibility test, we used a DNT target with four different functionalized surfaces and acquired direct electrical signals from each surface. With the combination of a highly specific peptide receptor and a multiple detection technique, we proposed a general platform using a multiple cantilever detection system. Also, a generic platform with multiple detection techniques could provide multitarget detection, which is essential for an electronic nose.

EXPERIMENTAL SECTION

Microcantilever Platform. We fabricated a cantilever arrays chip with four reaction chambers (microwell with 70 μL volume), as shown in Figure 1. Figure 1 shows a cantilever array chip that includes four cantilever units (Figure 1a), and a cantilever unit has three sensing cantilevers and one reference cantilever pattern (Figure 1b,c). Each microcantilever has the unique function of direct electrical detection via a piezoelectric thin film, as shown elsewhere.[5,23] The microcantilever, incorporating piezoelectric thin films, was composed of six multilayers, SiN_x/Ta/Pt/PZT/Pt/SiO_2, having a thickness of 2.18 μm, in order to achieve a simple self-actuating and self-sensing operation without the aid of optical components. The multiple-target cantilever sensor contains four microwells (four cantilever units) for individual surface functionalization. The reference cantilever pattern was designed for signal enhancement by completely obliterating the parasitic capacitance element. While the reference cantilever pattern has the same area as the cantilevers, it is structured on membranes; consequently they only produce the same capacitance, both off resonance and in resonance with the cantilever. We acquired signal enhancing (increasing SNR) by differential signal between the cantilever and the reference, since we were able to eliminate background capacitance and analyze inducing charge generated from the only mechanical vibration.

Peptide Receptor Immobilization. After preparing a 10/50 nm thick Cr/Au layer on the microcantilever (bottom side), we first cleaned the Au surface in a fresh piranha solution (a 4:1 ratio of H_2SO_4 (98.08%) and H_2O_2 (34.01%)) to remove any contaminants present on the gold surface and then rinsed with deionized water. DNT specific peptide was prepared using a customized solid phase peptide synthesis procedure on Fmoc-Rink amide MBHA resin (AnaSpec. Inc., San Jose, CA). The carboxylic acid-presenting self-assembled monolayers (SAMs) were prepared by immersing the microcantilevers into solutions of hydroxy-terminated tri(ethylene glycol)-alkanethiol(C11) and carboxy-terminated penta(ethylene glycol)-alkanethiol-(C11) at a ratio of 90:10 for 12 h. The monolayer was then treated with aminoethyl maleimide (50 mM in PBS, pH 7.4) and EDC (100 mM in PBS, pH 7.4) for 2 h, washed with absolute ethanol, and dried under a stream of nitrogen. The maleimide-presenting monolayer was incubated with peptides (10 mM in PBS, pH 7.4) for 12 h, washed with absolute ethanol, and stored at 4 °C. At the terminal end of the peptide, a cysteine was attached for immobilization. By immobilizing the DNT specific peptide through tri(ethylene glycol)-based SAMs formation, we expect selective DNT molecule detection in the gas phase.[24] From a phage display screening method,[25] we demonstrated Lys-Met-His-Thr-Ala-Ser-Leu-Ser-Gln-Pro-Leu-Met as the strongest DNT binding sequence.[7]

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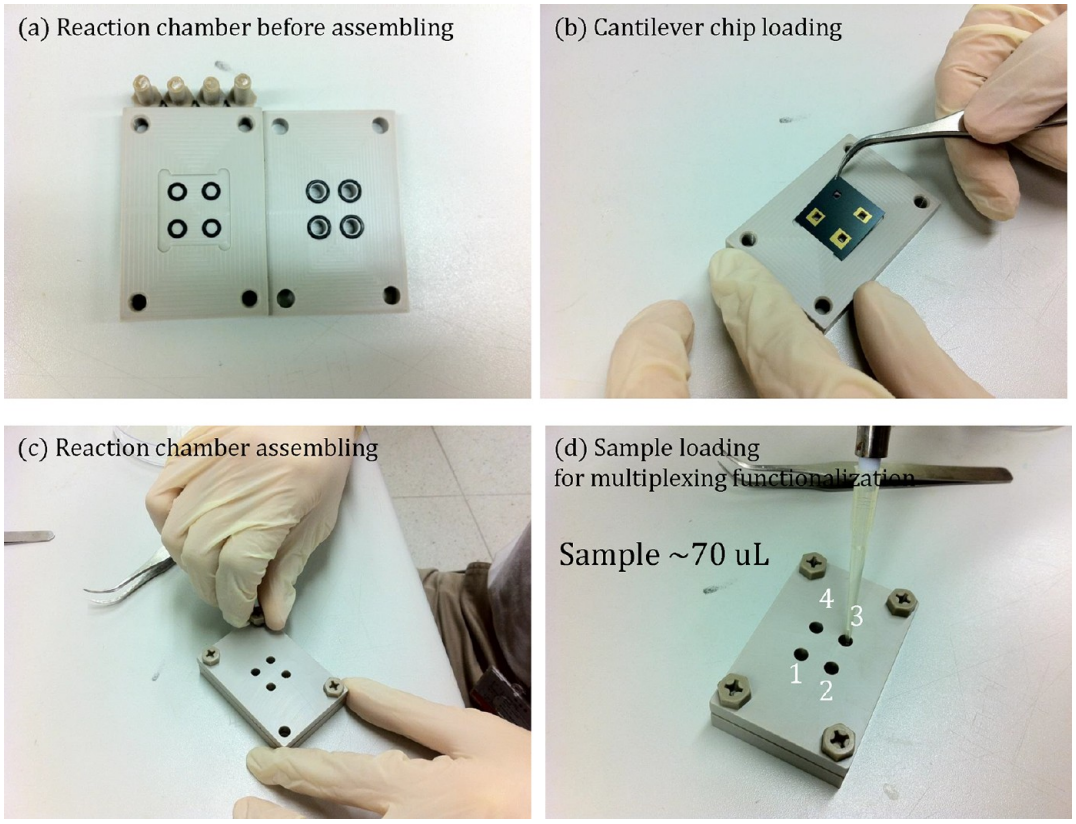

Figure 2. Sequential process of surface immobilization using the reaction chamber.

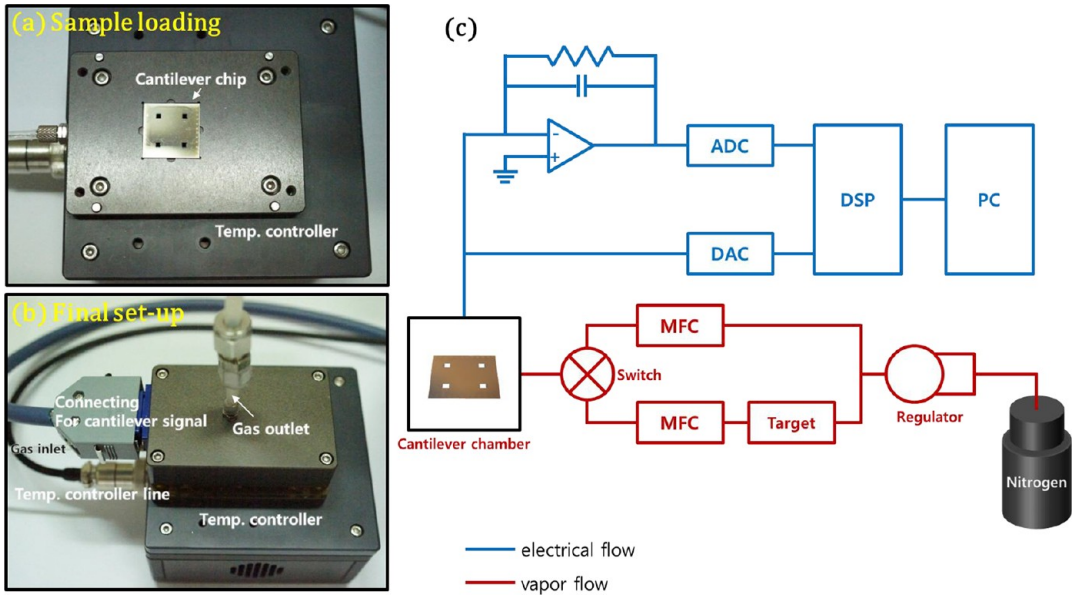

Figure 3. Measurement system: optical images of cantilever gas chamber of (a) before sample loading and (b) after sample loading and (c) gas flowing system.

Surface Treatment for Multiple Target Detection. We fabricated a special reaction chamber, as shown in Figure 2. The images in Figure 2 contain the sequential process of surface immobilization. We designed and prepared the reaction chamber with poly(ether ether ketone) (PEEK) materials and then loaded the cantilever chip. After assembling the reaction chamber, we injected the solutions for preparing multifunctionalized surfaces. By simply injecting chemicals using a micropipet directly on four microreaction chambers, we immobilized DNT specific peptide (HPNFSKYILHQRC; SP), DNT nonspecific peptide (TSMLLMSPKHQAC; NSP), and self-assembled monolayer (SAM). Cysteine of DNT specific and nonspecific peptide at the terminal was utilized to covalently anchored on the maleimide-presenting monolayer by Michael addition.[7] Also, one cantilever unit was prepared without an Au layer for a negative control. In order to

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immobilize the peptide with a specific sequence on the gold deposited 3 unit microcantilever surfaces, terminal modified ethylene glycol self-assembled monolayer (SAM) and maleimide were commonly used, and 70 μL volumes were injected into 3 microreaction chambers with a micropipet. Here, the bare cantilever surface without Au was not treated with chemical for surface immobilization. Importantly, one can utilize this reaction chamber for multitarget detection. For immobilizing DNT specific binding peptide, we injected a peptide with the His-Pro-Asn-Phe-Ser-Lys-Tyr-Ile-Leu-His-Gln-Arg-Cys sequence in the microreaction chamber no. 1. To check the specificity of DNT specific peptide, we prepared nonspecific binding peptide (Thr-Ser-Met-Leu-Leu-Met-Ser-Pro-Lys-His-Gln-Ala-Cys) and injected the chemical solutions into reaction chamber no. 2. To monitor the SAM's effects, we prepared the SAM surface on the Au coated cantilever using chamber no. 3. The cantilever unit prepared without Au was not functionalized and could be used as a negative control to monitor environmental changes, such as temperature and turbulence effects.

Measurement Setup. The optical images in Figure 3a,b show the sample loading process and final setup for the direct transduction of chemical interaction to electric signal, via the piezoelectric embedded cantilever. After surface functionalization of the multiwell microcantilever (see Figure 2), we disassembled the reaction chamber and then cleaned the surface with ethanol and DI-water. After the cleaning process, the multiwell microcantilever was located in the gas chamber. The gas chamber contains 20 electrical probes to acquire electrical signals between the top and bottom electrode from 16 cantilevers (16 probes for the top electrode and 4 probes for the bottom electrode). In general, mechanical sensors, such as cantilevers and bridges, tend to be affected by mechanical disturbance. To minimize mechanically induced resonant frequency shifts from the electric signal, we used a gas generator system, consisting of a nitrogen line, two mass flow controllers (MFC, TSC-D, MK Precision Inc.), an on/off solenoid valve, and a toggle switch (see more detailed information in ref 7). Moreover, monitoring of the bare cantilever without an Au layer could also provide information about the gas flow, gas induced temperature fluctuation, small pressure changes, gas density, and external mechanical vibration effects. Using the reference cantilever, we could also monitor and eliminate the response error due to physical adsorption by extracting multiple signals. We carried out all the measurements in the gas phase under atmospheric pressure and room temperature. To distribute gas flow uniformly, we designed a gas chamber with a vertical flow of DNT gas from top to bottom. Since the multiwell on the cantilever array chip was designed symmetrically (see Figure 1), the DNT gas from the bottom inlet flows through four open windows and is exhausted to the outlet gas line. We prepared 5 g of DNT particles in a 500 mL closed glass chamber at room temperature, and the equilibrium concentration of DNT gas was reported as 160 ppb at 20 °C. We precisely controlled the DNT gas with a mass flow controller (MFC). For measuring electrical signals from 16 cantilevers, we used an electrical measurement system equipped both with the analog-to-digital converter (ADC) connected with a charge amplifier and the digital-to-analog converter (DAC), which was processed using a digital signal processor (DSP) controlled by a PC (Cantis Co. Korea).

RESULTS AND DISCUSSION

In order to verify the drift effects with time, we monitored resonant frequency shifts of the cantilever with DNT SP, with flowing 50 sccm N_2 purging gas, as in Figure 4. When N_2 purge

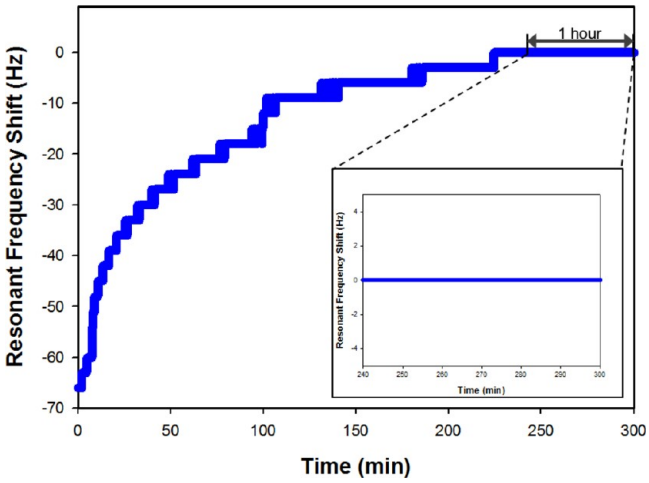

Figure 4. Resonant frequency shifts of cantilever with DNT specific peptide with flowing 50 sccm N_2 purging gas, showing the drift effects with time.

gas was delivered to the cantilever surface and flowed through the cantilever, we observed large positive shifts in resonant frequency (~70 Hz) for 200 min and then the frequency stabilized. Considering the large induced frequency shift with flowing N_2 purge gas, it is reasonable to infer that the dry N_2 gas removed the absorbed water molecules from the functionalized cantilever and changed the surface temperature of the cantilever as well. To start measurement under a stabilized condition, we carried out DNT gas injection after a 5 h N_2 purging process, when resonant frequency response with time had stabilized.

To confirm the ability of multiple target detection, we simultaneously monitored the resonant frequencies of cantilevers with four different surfaces (Figure 5). We monitored the resonant frequency response by injecting 100 sccm DNT, as in Figure 5a, while we acquired a response with a 50 sccm DNT injection, as in Figure 5b. Four different surfaces were prepared with DNT SP, DNT NSP, SAMs, and a bare cantilever. The frequency shifts in a cantilever with DNT SP showed a sharp decrease in frequency, while the resonant response with the DNT NSP, SAMs surfaces, as well as the bare cantilever reveals relatively small changes of frequency, both in Figure 5a,b. In previous work, we showed the 100 ppb detection of DNT as well as DNT SP's ability of selectivity using a control experiment, both with toluene (200 ppm) and nitro-toluene (200 ppm).[7] While the concentration of toluene (200 ppm) and nitro-toluene (200 ppm) is 3 orders larger than that of DNT, frequency shifts of toluene (200 ppm) and nitro-toluene (200 ppm) are relatively small (~3 Hz), compared with ~12 Hz in DNT gas. However, simultaneous detection of targets and references for the multitarget detection has not yet been demonstrated in the previous work. We demonstrated the ability of multiple target detection with four different surfaces. In gas detection, one is usually faced with difficulties in the control of gas concentration. Gas concentrations have been affected by environmental temperature, since they have strong temperature dependency. For example, the DNT concentration

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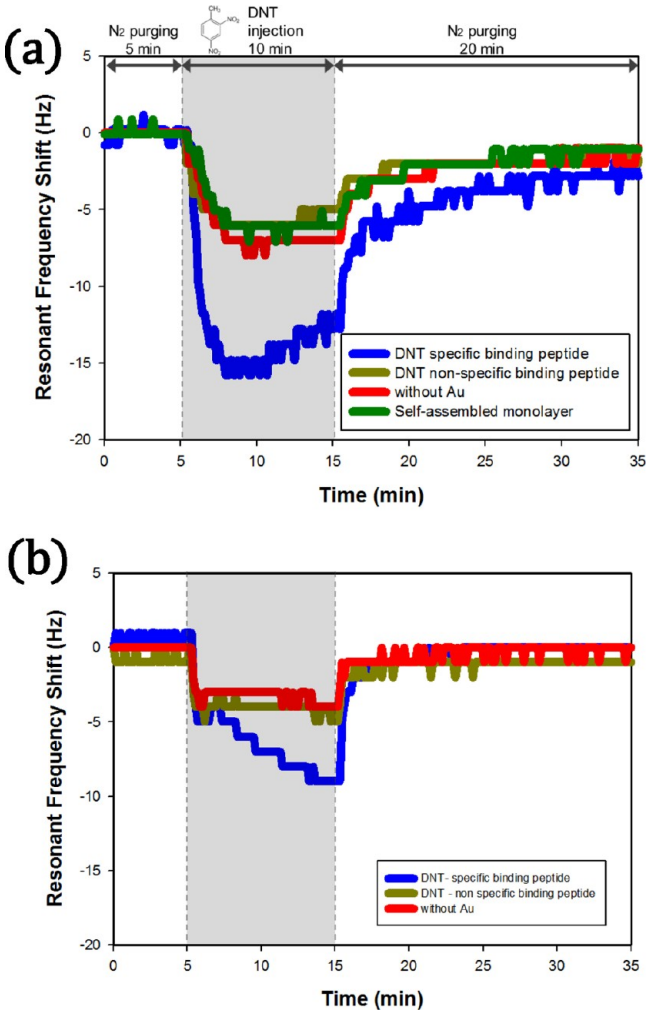

Figure 5. Multiple signals with flow velocity of (a) 100 sccm and (b) 50 sccm. The DNT molecule was a limited source in both cases.

has been reported as 490 ppb at 30 °C, while as 160 ppb at 20 °C. Small temperature variance also affects a cantilever's resonant frequency. We acquired resonant frequency shifts with temperature as +5 Hz/C, because of differences in the coefficient of temperature expansion (CTE) of a multilayered cantilever. Moreover, one should consider mechanical damping with medium density and viscosity, as well as temperature variance, when one injects and changes gas (medium). To solve the problems, we monitored the reference signal from the cantilever array chip. The bare cantilever could provide the variation of CTE as well as the effect of the medium. Also, by extracting the SP and NSP cantilevers, one can acquire information of specific binding effects.

In Figure 5a, we observed that the resonant frequency decreased dramatically in the first 2.5 min, saturated for the following 2.5 min, and then slightly increased for the last 5 min. We injected the DNT gas with a 100 sccm flow rate, with flowing through a prepared DNT particle gas chamber. The DNT gas was expected as a limited source, since the volume of DNT gas was saturated as a constant volume and concentration, as in both parts a and b of Figure 5. DNT gas delivered to a cantilever chamber by N_2 flowing could be a column shape during N_2 blowing, so that fast flowing N_2 with 100 sccm (Figure 5a) could lead to fast delivery of limited DNT gas and consequently lead to depletion of gas sources after 5 min. Meanwhile, DNT gases delivered slowly with 50 sccm could take at least 10 min for complete DNT gas delivery (Figure 5b). In Figure 5a, the initial sharp decrease of binding curve corresponds with the interaction between DNT gas column and DNT specific peptide. The decrease in resonant frequency was saturated in 2 min, and the resonant frequency increased after 7 min of DNT gas injection. When a limited source of DNT passed through the cantilevers, N_2 purging gas flowed with forced convection, which expedited the main desorption process based on diffusion. In contrast to frequency shifts from 100 sccm (Figure 5a), the results from 50 sccm (Figure 5b) showed ever-decreasing resonant frequency shifts with 10 min DNT gas detection.

In Figure 6, we show the differential signal between DNT SP and DNT NSP. The differential signal (blue line in Figure 6)

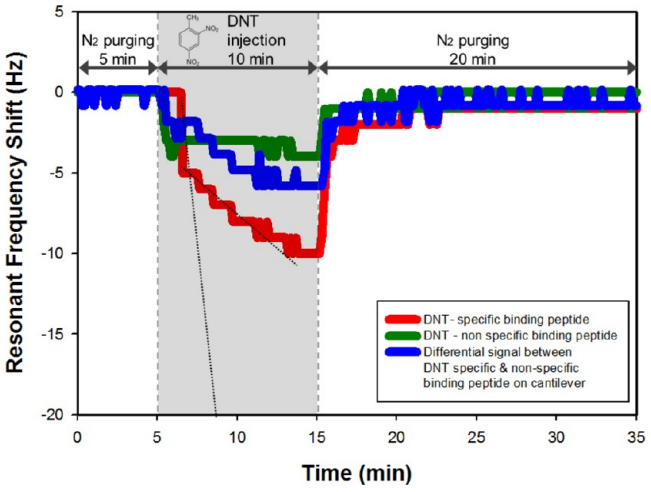

Figure 6. Differential signal providing the resonant frequency shifts from bindings, regardless of environmental changes.

was calculated by extracting NSP from the SP signal, and consequently we observe pure information from the specific bindings via resonant frequency shifts, without inevitable thermal noise, vibration noise, and humidity effects. The resonant response from the SP immobilized cantilever (the red line in Figure 6) was ever-decreasing with DNT gas injection for 10 min, while one from NSP (green line in Figure 6) dropped and stabilized with DNT gas injection. Differential values in resonant frequency response give us clear information of the reaction kinetics between SP and DNT gas molecules in various environmental conditions.

■ CONCLUSIONS

We have demonstrated a platform that provides a multitarget detection via a simple real-time electrical readout. A multiple detection technique in a nanomechanical cantilever is essential for electronic nose systems. We fabricated four microwell structures in a chip. By using four different functionalized surfaces, we demonstrated the ability of binding of DNT targets with functionalized cantilever surfaces. Generally, microcantilever sensors are very sensitive, so that detection using microcantilever is usually influenced by small environmental changes. By utilizing the differential signal of SP and NSP, we acquired resonant signals, which contained the genuine information of specific binding, by eliminating thermal noise, vibration noise, and humidity effects. This platform, with its simplicity and efficient capability for multiple detections, could

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be a generic and powerful tool for electronic nose systems. Furthermore, the use of a peptide receptor can significantly improve the selectivity of nanomechanical cantilever sensors used for olfactory systems.

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Notes

The authors declare no competing financial interest.

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