Differentiation of Complex Vapor Mixtures Using Versatile DNA-Carbon Nanotube Chemical Sensor Arrays

Nicholas J. Kybert[*], Mitchell B. Lerner[*], Jeremy S. Yodh[*], George Preti[**,***] and A. T. Charlie Johnson[*]

[*] Department of Physics and Astronomy and Nano/Bio Interface Center,
University of Pennsylvania, Philadelphia, Pennsylvania 19104, USA, cjohnson@physics.upenn.edu
[**] Monell Chemical Senses Center, Philadelphia, Pennsylvania 19104, United States
[***] Department of Dermatology, School of Medicine, University of Pennsylvania, United States

ABSTRACT

Vapor sensors based on functionalized carbon nanotubes (NTs) have shown great promise, with high sensitivity conferred by the reduced dimensionality and exceptional electronic properties of the NT. Critical challenges in the development of NT-based sensor arrays for electronic nose systems include the demonstration of reproducible fabrication methods and functionalization schemes that provide high chemical diversity to the resulting sensors. Here, we outline a scalable approach to fabricating arrays of vapor sensors consisting of NT field effect transistors functionalized with single-stranded DNA (DNA-NT). DNA-NT sensors were highly reproducible and target analytes were detected even in large backgrounds of volatile interferents. DNA-NT sensors were able to discriminate between highly similar molecules, including structural isomers and enantiomers. The sensors were also able to detect subtle variations in complex vapors, including mixtures of structural isomers and mixtures of many volatile organic compounds characteristic of humans. This work paves the way for incorporation of DNA-NT sensor arrays in "electronic nose"-type systems.

Keywords: vapor sensor, carbon nanotube, field effect transistor, DNA, electronic nose

1 INTRODUCTION

NT-based sensors have been demonstrated for diverse environmental, defense and medical applications.[1,2,3,4,5] Chemical modification of the NT surface is a powerful method to influence the interaction strength between the NT and analyte molecules and thereby improve the device sensitivity and specificity. The promise of DNA as a functionalizing agent for NT-based vapor sensors is based on its complex but completely controlled chemistry, which provides affinity for a wide variety of analytes and enables control of sensor responses through choice of the DNA sequence. In previous work,[5,6] DNA-NT transistors based on individual, CVD-grown nanotubes were used to detect single analytes at concentrations as low as a few ppb and to distinguish between highly similar compounds, including structural isomers and enantiomers. In this work,[7] we report the fabrication of NT FET arrays using commercially available solutions enriched in semiconducting NTs and NT-compatible photolithographic fabrication methods.[8] Arrays of NT FETs had very good device-to-device reproducibility and 90 % yield of useful devices. DNA-NT sensors demonstrated highly favorable sensing properties, very similar to those reported for sensors based on single NTs.[5] They showed reproducible responses to single analytes that could be fit to predictions from equilibrium thermodynamics. These responses were almost identical when the target was presented in a background with a high concentration of compounds known to block human olfaction. DNA-NT sensors were found to provide differential responses to highly similar compounds, including enantiomers of limonene, and three distinct forms of pinene, a compound with two structural isomers, each with two enantiomeric forms. DNA-NT devices were also tested against vapor mixtures to provide a more realistic assessment of their potential for use in complex environments and medical diagnostics based on volatile biomarkers. The sensors were found to respond to complex mixtures of volatiles characteristically emitted by humans and to be sensitive to slight alterations of the mixture.

2 RESULTS AND DISCUSSION

DNA-NT vapor sensors were fabricated by modifying an established scalable process based on commercial NT solutions.[9,10] To promote adhesion of NTs to the substrate, the surface was pretreated with a reproducible, uniform monolayer of 3-aminopropyltriethoxysilane (APTES), deposited using atomic layer deposition (Savannah 200, Cambridge Nanotech), with surface pretreatment by introduction of H_2O vapor to increase the concentration of hydroxyl groups. Optimum values of the concentration of the NT solution (NanoIntegris, Isonanotubes-S 98%) and the incubation time were found to be 10 mg/mL and 20 min, respectively. current-gate voltage characteristics (I-Vg) of the devices were low-noise with good semiconducting behavior (95% functional device yield with 90% having an on/off ratio exceeding 20; see Fig. 1c). The measured distribution of threshold voltages across devices in a typical array (1.5 V ± 1.8 V) was indicative of low doping and high process reproducibility.

Four different DNA oligomers were used in this work:
Seq1 5' GAG TCT GTG GAG GAG GTA GTC 3'

Seq2 5’ CTT CTG TCT TGA TGT TTG TCA AAC 3’
Seq3 5’ GCG CAT TGG GTA TCT CGC CCG GCT 3’
Seq4 5’ CCC GTT GGT ATG GGA GTT GAG TGC 3’

To confirm the formation of a nanoscale DNA layer on the NTs, AFM images were taken of the same region of a NT film before and after DNA functionalization. The data showed a height increase of 0.56 ± 0.2 nm after application of DNA, similar to previous measurements of self-assembled DNA layers on graphene sheets. The substrate height and roughness remained unchanged, indicating DNA deposition was predominantly onto the NTs.

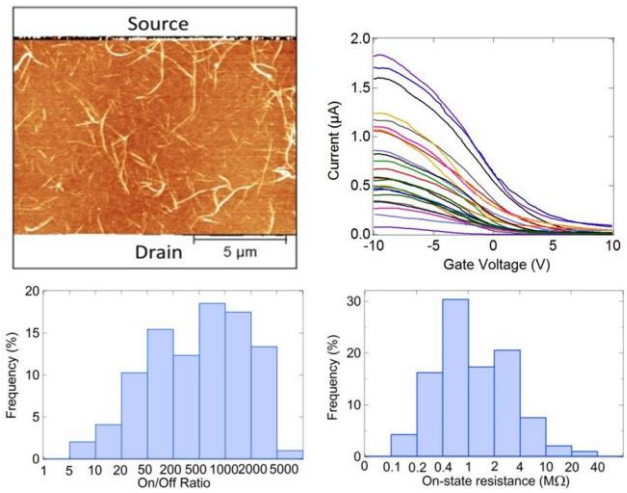

Figure 1: a) AFM image of a typical device showing a sparse nanotube network between electrodes. Z-scale is 4 nm. b) I(V_g) curves of a representative set of 25 devices, with V_{DS} = 100 mV. c) Histogram of on/off ratios shows large on/off ratios. d) On-state resistance histogram shows a tight spread, implying good reproducibility across devices.

Fig. 2a) shows sensor responses to dimethylsulfone (DMSO2), a compound found in human body fluids including skin secretions and volatiles collected above human skin[11,12] which has been preliminarily identified as a volatile biomarker of basal cell carcinoma[13]. The device responses were rapid (seconds), reproducible in time and across devices, and they returned to baseline upon flushing with clean air without need for sensor refreshing. The data are well fit by the prediction of a Langmuir-Hill model of analyte binding dynamics,

$$\frac{\Delta I}{I_0} = A\frac{C^n}{C^n + K_a^n} + Z \qquad (1)$$

where C is the analyte concentration, A is the magnitude of the response when all binding sites are occupied, K_a is the concentration at which half the maximum response is seen and n is the Hill coefficient describing cooperativity of binding. Remarkably, the response magnitude remains unchaged when a large amount of an interferent chemical, cis-3-Hexen-1-ol, which is known to inhibit mamalian olfaction,[14] is introduced into the environment. The lack of effective blocking of the response in these two cases presumably derives from the fact that the nature of DNA as a “receptor” for volatile compounds differs significantly from that of human olfactory receptor proteins.

We also tested the ability of the DNA-NT sensors to destinguish between extremely similar molecular structures, including enantiomers of limonene and structural isomers of pinene, a compound that has two structural isomers, each of which has a pair of enantiomers. The averaged responses

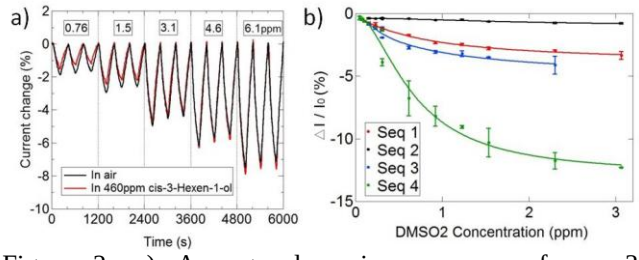

Figure 2: a) Average dynamic responses of seq 3 functionalized devices to $DMSO_2$ in both clean air (black) and 460 ppm cis-3-Hexen-1-ol show high reproducibility between exposures and nearly identical responses in the presense of the blocker. b) b) Average responses of DNA-NT based on four different DNA oligomers to DMSO2 and the corresponding Langmuir-Hill fits.

of DNA-NT based on Seq1 to the enantiomers of limonene are shown in Fig. 3a; there is clear discrimination between these highly similar molecules. The standard Langmuir-Hill fit worked well for the data for D(+) limonene but poorly for L(-) limonene. Responses for L(-) limonene showed anomalous behavior with a positive response at low concentrations that crossed over to a negative response for concentrations exceeding ca. 40 ppm. This behavior is consistent with the presence of two distinct types of binding sites for limonene, one that leads to a positive response for both enantiomers and one that leads to positive and negative responses for the D(+) and L(-) enantiomers, respectively. Given that previous experiments from our lab, which employed DNA-NT based on Seq1 and single NTs grown by CVD, also showed positive responses for the D(+) enantiomer and negative responses for the L(-) form, we suggest that the binding site that distinguished between enantiomers was associated with the DNA, while the other site corresponded to binding of the analyte to junctions between NTs in the network.

Isomers of pinene were also clearly distinguished by DNA-NT sensors (Fig. 3b); both the double bond location and the handedness of the pinene molecule affected the sensor responses, with DNA-NT based on Seq3 showing greater differentiation than those based on Seq1. As a further test of the differentiation power, DNA-NT devices based on Seq1 and Seq3 were tested against mixtures of the α(-) and β(-) structural isomers of pinene (Fig. 3c). The averaged responses of 5 devices based on Seq3 provided the ability to resolve composition changes of approximately 5-10% in a vapor where the total pinene concentration was held fixed at 130 ppm. Devices based on Seq1 showed less discrimination power for the mixtures of α(-) and β(-)

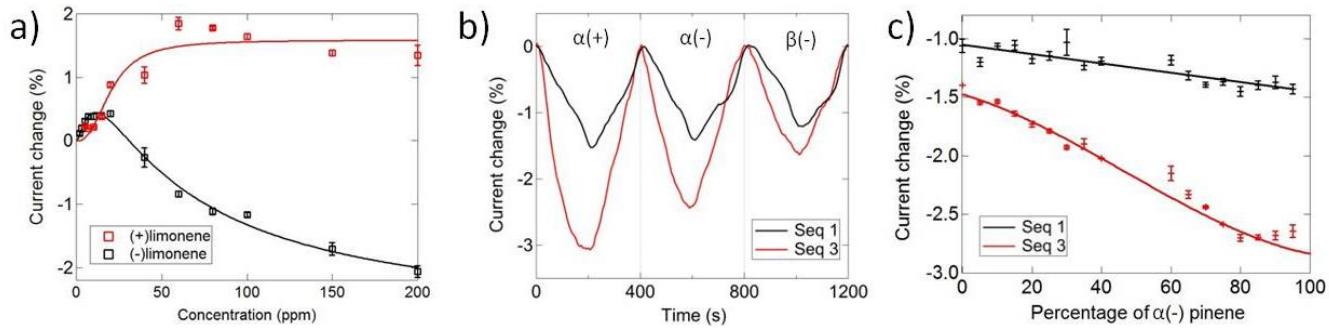

Figure 3: a) DNA-NT devices based on Seq1 clearly distinguish limonene enantiomers. The responses to D(+) limonene (red data) are well fit by a simple Langmuir-Hill equation, while responses to L(-) limonene (black data) require a two-component fit, suggesting the existence of two distinct binding sites. b) Responses of DNA-NT based on Seq 1 (black data) and Seq 3 (red data) to pulses of α(+),α(-) and β(-) pinene at a concentration of 130 ppm. The responses depend on both the location of the double bond and the handedness of the molecule. c) The responses of DNA-NT based on Seq1 and Seq3 decrease as the analyte is adjusted from pure α(-) pinene to an α(-)/β(-) mixture to pure β(-) pinene.

pinene than those based on Seq 3, consistent with their respective responses to the neat analytes (Fig. 3b).

An essential characteristic of biological olfactory systems is the ability to differentiate between very similar *complex mixtures* of volatile compounds. Experiments to test the ability of DNA-NT devices in this domain were based on a mixture of 17 organic compounds, many of which are volatile and found in non-axillary skin sweat.[11] These were dissolved in physiological saline (see Table 1). DNA-NT devices were exposed to the headspace vapor of the original "parent" mixture and also to that of "spiked" mixtures where one component was increased in concentration by a factor of 2-10. "Spiked" mixtures were based on compounds that were prevalent in the mixture (acetic acid) and those found in trace amounts (stearic acid and nonanal), with widely varying vapor pressures.

Compound	Concentration, mg/mL	Vapor Pressure, Torr (@ 20 C)
Acetic Acid	0.67	3.0
Lactic Acid	0.66	0.08
Glycerol	0.17	1 @ 125 C
Stearic Acid	0.03	1 @ 174 C
Acetoin	0.05	2.69
Propanoic Acid	0.09	2.9
Isobutyric Acid	0.01	1.5
Butyric Acid	0.45	0.43
Isovaleric Acid	0.01	0.38
2-Methylbutyric Acid	0.01	0.5
Isocaproic Acid	0.01	N/A
4-Methyl-phenol	0.05	1
Phenol	0.01	0.36
Dimethylsulfone	0.05	N/A
Nonanal	0.01	0.26
Indole	0.03	0.03
Squalene	0.20	2 @ 240 C

Table 1: Components of the Parent Mixture Used in the Experiments

The concentrations of various components in the headspace of the mixtures were not measured. However, estimates were made using Raoult's law, which assumes that the concentration of a mixture component is the product of the vapor pressure of the component and its molar fraction in the solution. For the parent mixture this yielded 793 ppb for acetic acid and 0.43 ppb for nonanal. Stearic acid is a solid at room temperature with a very low vapor pressure (see Table 2). Although no precise estimate could be formulated, the expected concentration in the headspace of the parent mixture would surely be well below 1 ppb. For a spiked mixture, these concentrations were multiplied by the appropriate spiking factor (*i.e.*, 2x, 5x, or 10x).

The spiked mixtures were exposed to DNA-NT devices concurrently with the standard mixture and the sensor responses ($\Delta I/I_0$) were recorded. DNA-NT devices were typically, but not always, found to provide strong differential responses between the parent mixture and spiked mixtures. Responses of DNA-NT based on Seq3 were very sensitive to the concentration of nonanal in the mixture (Fig. 5a). We note that the estimated concentration of nonanal in the vapor ranged from 0.43 – 4.3 ppb. Differential response of DNA-NT devices to the "parent" and "spiked" mixtures depended on the identity of the DNA oligomer. For example, responses of DNA-NT based on Seq1 to the "parent" mixture and a mixture "spiked" by 10x with stearic acid were nearly identical, while responses of DNA-NT based on Seq3 showed clear differentiation between these two mixtures (Fig. 5b, red and green data, respectively). The concentration of stearic acid in the vapor is not precisely known but is almost certainly at the level of a few ppb or lower. The implication is that stearic acid does not significantly bind to Seq1 in the presence of all the other VOCs but that it does bind strongly to sequence 3. Furthermore, a very rich data set was obtained by considering DNA-NT differential responses to the headspace vapor of the "spiked" mixture at various dilutions with clean air. As seen in Fig. 5c, the dependence of the differential responses of DNA-NT based on Seq4

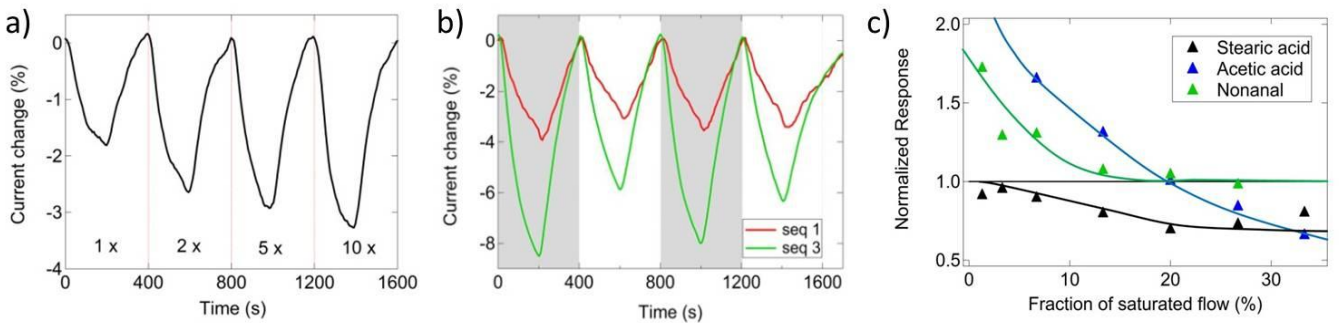

Figure 4: a) DNA-NT device based on Seq3 provides clear differential responses between the "parent" mixture and mixtures "spiked" with nonanal by factors of two, five and ten (2x, 5x, 10x, respectively). b) Responses of DNA-NT based on Seq1 (red data) and Seq3 (green data) to 33% saturated vapor of the "parent" mixture (gray background) and a "spiked" mixture with 10x increased concentration of stearic acid (white background). Devices based on Seq3 show a strong differential signal to the two mixtures while the differential signal for DNA-NT based on Seq1 is weak. c) Responses of DNA-NT based on Seq4 to diluted streams of headspace vapor of "spiked" mixtures, normalized to the response to the parent mixture. In each case, the named component is spiked by a factor 10x compared to its concentration in the parent solution. Markers are experimental data, and the solid lines are guides to the eye.

with dilution depends on the identity of the "spiked" component. Consequently, real world mixtures could potentially be identified by comparison to a standard mixture using DNA-NT sensors. By measuring response deviation as a function of dilution, it could be possible to identify exactly which compound in the mixture has been altered and by how much its concentration has changed.

3 CONCLUSION

In summary we have demonstrated a facile, potentially scalable method for fabricating DNA-NT vapor sensors that could enable their use in sensor arrays suitable for incorporation into an electronic nose system. We tested device responses against individual VOC analytes characteristic of humans and against complex mixtures that more closely resemble "real-world" samples. DNA-NT sensors showed excellent reproducibility, they responded within seconds to parts per billion concentrations, and their responses were in good agreement with predictions of equilibrium thermodynamics. Devices were able to differentiate between analytes with very similar molecular structure (*i.e.*, enantiomers and structural isomers), they were able to detect target analytes in a large background of an interfering VOC, and they could discriminate subtle changes in complex VOC mixtures. The use of DNA as the functionalizing agent holds the possibility that arrays of hundreds or thousands of individual sensors could be fabricated on a single chip and functionalized with a large number of different DNA oligomers. As each sequence has its own set of characteristic responses to a large number of various analytes, this approach should allow analytes to be detected and distinguished at relevant concentrations across many applications, including deducing chemical composition in an unknown environment or disease diagnosis from VOCs.

4 REFERENCES

1 D. R. Kauffman and A. Star, Angew Chem Int Edit **47** (35), 6550 (2008).

2 C. Zuniga, M. Rinaldi, S. M. Khamis, A. T. Johnson, and G. Piazza, Appl Phys Lett **94** (22), 223122 (2009).

3 E. S. Snow, F. K. Perkins, E. J. Houser, S. C. Badescu, and T. L. Reinecke, Science **307** (5717), 1942 (2005).

4 A. Star, J. C. P. Gabriel, K. Bradley, and G. Gruner, Nano Lett **3** (4), 459 (2003).

5 S. M. Khamis, R. A. Jones, A. T. C. Johnson, G. Preti, J. Kwak, and A. Gelperin, Aip Adv **2** (2), 022110 (2012).

6 C. Staii, A. T. Johnson, M. Chen, and A. Gelperin, Nano Lett **5** (9), 1774 (2005).

7 N. J. Kybert, M. B. Lerner, J. S. Yodh, G. Preti, and A. T. Johnson, ACS Nano DOI:10.1021/nn400359c (2013).

8 S. M. Khamis, R. A. Jones, and A. T. C. Johnson, Aip Adv **1** (2), 022106 (2011).

9 C. Wang, J. L. Zhang, K. M. Ryu, A. Badmaev, L. G. De Arco, and C. W. Zhou, Nano Lett **9** (12), 4285 (2009).

10 S. J. Choi, C. Wang, C. C. Lo, P. Bennett, A. Javey, and J. Bokor, Appl Phys Lett **101** (11), 112104 (2012).

11 M. Gallagher, J. Wysocki, J. J. Leyden, A. I. Spielman, X. Sun, and G. Preti, Brit J Dermatol **159** (4), 780 (2008).

12 X. N. Zeng, J. J. Leyden, A. I. Spielman, and G. Preti, J Chem Ecol **22** (2), 237 (1996).

13 G. Preti, M. Gallagher, S. S. Fakharzadeh, C. J. Wysocki, J. Kwak, J. Marmion, H. Ozdener, C. J. Miller, C. D. Schmults, A. I. Spielman, X. M. Sun, and S. Chachkin, Chem Senses **33** (8), S138 (2008).

14 S. Serra, C. Fuganti, and E. Brenna, Trends Biotechnol **23** (4), 193 (2005).