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Analysis of Sweat Simulant Mixtures using Multiplexed Arrays of DNA-Carbon Nanotube Vapor Sensors

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Abstract

Carbon nanotube (NT) based electronic vapor sensors were tested against synthetic sweat solutions, consisting of 13 volatile organic compounds (VOCs) in saline, in order to probe the device ability to analyze and differentiate vapors derived from complex biological samples. Arrays of up to 56 NT devices each were fabricated and functionalized with single stranded DNA to increase sensitivity and selectivity. DNA/NT devices were able to differentiate changes as small as 50% in a compound with estimated concentration in the vapor at part-per-billion levels, in a complex vapor background that contained the thirteen VOCs. This sensor class has vapor response times on the order of ten seconds, and is reusable and self-refreshing. The fabrication process is scalable, and sensor arrays are compact compared to traditional analysis equipment such as gas chromatography/mass spectrometry (GC/MS). The detectable differences among the simulated sweat mixtures were on the same scale as person-to-person variations in VOCs reported by others previously, demonstrating that DNA/NT vapor sensors show great promise for odor-based chemical biometric applications.

Introduction

Humans emit a variety of volatile organic compounds (VOCs) in urine, sweat and other bodily fluids. A large body of literature now shows that an individual's emitted odors form a unique chemical profile [1-3]. For example, studies have shown that VOC profiles possess characteristic components that depend on diet [4], gender [5], age [6] and ethnicity [3,7,8]. Sweat composition is also known to vary under stress, so much so that studies have shown that chemosignals of stress influence social judgments [9]. Olfactory evaluation can be an important part of clinical examination [10] and certain volatile compounds, emitted through the skin and other bodily fluids, have been shown to be possible biomarkers for a whole host of diseases and disorders [11-13]. However, this information, contained in the individual's volatile signature, remains essentially untapped. Techniques to quantitatively analyze VOC profiles in sweat and other bodily fluids could find applications in disease diagnostics, health and wellbeing monitoring, and even for detection, identification or tracking of individuals.

Gas chromatography/mass spectrometry (GC/MS) and related techniques have been the most widely used methods of analyzing complex VOC profiles to date. These are "gold standard" techniques for researching unknowns, given the methods' ability to separate, identify and quantify compounds in complex mixtures of volatile compounds. However, there remains a need for technologies that can perform similar analysis quickly and efficiently on mixtures of known VOCs without the cost and bulk associated with current commercial techniques such as GC/MS, either desktop or smaller sized.

Vapor sensors based on low-dimensional carbon nanomaterials, such as graphene and carbon nanotubes (NTs), have been shown to have many of the required characteristics for such a task. These materials combine a favorable 'all-surface' geometry that maximizes the sensor's exposure to the vapor sample with highly sensitive electronic properties that are strongly affected by the local charge and molecular environment. Furthermore, their highly ordered surface structures allow for controlled chemical functionalization, including via covalent and π- π stacking interactions. Surface modifications have included decoration with metals [14,15] and metal oxides [16], polymer coatings [17], atomic doping [18] and functionalization with biomolecules [19], in particular single-stranded DNA [20-23]. The advantages of DNA include its straightforward π- π stacking interaction with NTs and its complex yet controlled chemical structure, which causes self-assembled DNA layers on NTs to have binding sites for a wide array of volatile molecules. Conductance responses of the sensors upon exposure to analyte vapors are DNA-sequence dependent and an almost

unlimited number of potential DNA sequences are available. DNA is also sufficiently inexpensive to be incorporated in large-scale device fabrication.

Previous studies on electronic vapor sensors based on DNA-functionalized carbon nanotubes have demonstrated the ability of these sensors to detect target analytes down to parts-per-billion level concentrations. These sensors can also discriminate between very similar molecules, such as homologous series of linear aldehydes and carboxylic acids, structural isomers and enantiomers [20,22]. The device sensitivity is also maintained if the target is in a background of other, chemically similar, VOCs. Initial experiments on complex vapors from dilute mixtures of volatile compounds showed that sensors could distinguish changes in the mixtures [22].

In this work, we demonstrate that arrays of DNA/NT vapor sensors have sufficient detection and discrimination power to provide information on VOC contents of extremely similar synthetic axillary sweat mixtures. These synthetic sweat solutions are based on previous studies of Zeng et al. [5,24] and consist of 13 of the characteristic odiferous compounds diluted to the average levels found in a group of males' axillary sweat. Headspace vapor from synthetic sweat solutions was pulsed into a chamber containing DNA/NT vapor sensors, and sensor responses to the VOCs were observed at the initial headspace concentration and when diluted by up to an order of magnitude with clean air. A series of very closely related solutions was prepared by changing the concentration of one of the components (either 9 decenoic acid or 3-methyl-2-hexenoic acid) to a value in the range 0-500% of the starting concentration. The sensors demonstrated the ability to distinguish solutions between which the altered component's concentration differed by as little as 50%, while all other 12 components remained fixed. These differences are smaller than typical person-to-person variations in VOC profiles in sweat reported by other groups, where the concentrations of different compounds vary by anywhere between a factor of 3 and more than an order of magnitude [1-3]. Consequently, these results show that DNA/NT sensors could be appropriate for a variety of next-generation odor-based chemical biometrics tests with applications in determining the health, wellbeing or identity of individuals based on volatile signatures.

Material/ Methods

DNA/NT array fabrication:

The procedure was similar to previous experiments [22]. Many arrays of 56 devices each were fabricated in parallel on Al_2O_3 coated Si/SiO_2 wafers by first using photolithography/metallization to produce Cr/Au electrodes and then drop casting semiconducting-enriched (98%) nanotube solution (NanoIntegris Inc., Boisbriand, Quebec, Canada) onto the chips. Careful washing, cleaning, and annealing steps were performed to remove surfactants and ensure good electrical contact between the NTs and gold electrodes [25]. Finally, DNA functionalization was performed by pipetting 100μM DNA solution onto the devices and allowing the DNA strands to diffuse to and bind onto the sidewalls of the NTs. The DNA strands bind via the π- π stacking interaction between the DNA bases and the NT surface [26,27]. After 30 minutes the DNA solution was blown off the chip with compressed nitrogen gas, removing all unbound DNA, and the devices were ready to use. Four different randomly selected DNA sequences (Invitrogen, Carlsbad, CA) were used in this work, as shown in Table 1. These sequences are not specifically engineered to have certain binding sites. However, earlier experiments indicated that DNA/NT devices based on complex DNA sequences show higher sensitivities than devices based on simple sequences (e.g. A_{21}) [20].

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'
Seq8	5' AGT TCG GCA TGT GGA AAC TCC TTC 3'

Table 1: DNA sequences used in this work

Synthetic sweat

The composition of the sweat solutions was guided by previous studies [5,24]. The male donors (N=6) who were used to create the pooled, male axillary extracts analyzed by Zeng et al. also donated six separate axillary pads which were pooled by individual, extracted and analyzed separately. For each subject, the components constituting the characteristic axillary odorants were quantified using standard curves made from both commercially available and synthesized compounds (e.g., E-3-methyl-2-hexenoic acid). The amount of 3-hydroxy-3-methyl-hexanoic acid was estimated from the study of Natsch et al. [28].

The mean amounts of each compound found in the six male donors were used to create the respective concentrations in the 'standard' sweat mixture used in this study; these are shown in Table 2. The components were obtained commercially from Sigma Aldrich Co. (St Louis, MO) unless otherwise stated. 3-Hydroxy-3-methyl-hexanoic acid (3H3MH), was obtained from PharmBlock, Inc. (Middleton, NY). Samples of E-3-methyl-2-hexenoic acid (3M2H) were obtained by synthesis [5]. The compounds were dissolved in deionized water by shaking and gentle heating. Finally sodium chloride was added to the solutions until they matched physiological saline levels of 9g/L.

Compound	Concentration (mg/L)	Vapor Pressure (Torr)
Hexanoic acid	35	0.18
4-Ethylpentanoic acid	0.8	0.058
2-Ethylhexanoic acid	5	< 0.01
Heptanoic acid	16	0.011
E-3-Methyl-2-hexenoic acid	100	0.105 (est.)
Octanoic acid	42	0.02
7-Octenoic acid	11	0.0115
Nonanoic acid	55	1.7 x 10-3
4-Ethyloctanoic acid	1	0.0018
8-Nonenoic acid	5	0.003 (est.)
Decanoic acid	61	3.7 x 10-4
9-Decenoic acid	9	0.018
3-Hydroxy-3methyl-hexanoic acid	15	0.001

Table 2: Components of the standard sweat solution and their respective concentrations and vapor pressures.

Vapor delivery and sensor array readout

Vapors were delivered to the arrays of DNA/NT devices using a lab-built system, described previously [22]. A stream of clean, dry air from a cylinder of compressed hydrocarbon-free 'zero' air (Airgas LLC, Malvern, PA) was passed through a bubbler containing either a pure VOC or a sweat simulant and then diluted with streams of clean, dry air and clean, humidity-saturated air. By controlling the ratios of the three flows and mixing them before flowing through the sample chamber, the bubbler headspace vapor could be diluted as desired. The vapor samples were passed across the sensors for 2 minutes, after which the chamber was flushed with clean air for two minutes and a new measurement was taken in the same fashion. The humidity level of the sample chamber was held fixed at 50% unless otherwise noted, while the total flow rate through the sensing chamber was held fixed at 1000 sccm. A picoammeter (Keithley 6485) and multiplexing switching matrix (National Instruments PXI 1033) were used to read the current flow through the devices sequentially, measuring the whole array approximately every two seconds.

Results and discussion

After fabrication, devices were characterized by atomic force microscopy and electrical transport measurements, as shown for a typical device in Figure 1. Devices consisted of 1-3 μm long single-wall carbon nanotubes, joined together in sparse networks to span the 10 μm gap between the electrodes. The devices showed reproducible p-type electronic behavior, with ~ 95% of the devices exhibiting on-off ratios exceeding 20. As the sensor response mechanism for acids is typically chemical gating by negatively charged deprotonated molecules, an increase in the device current upon exposure to the sweat stimulant vapor was expected. To compare devices with different on-state currents, the sensor response is reported as the change in the current, $\Delta I/I_0$. The gate voltage was held fixed at -8 V for all vapor-sensing measurements to maintain a high current level and transconductance, with the goal of maximizing the signal-to-noise ratio.

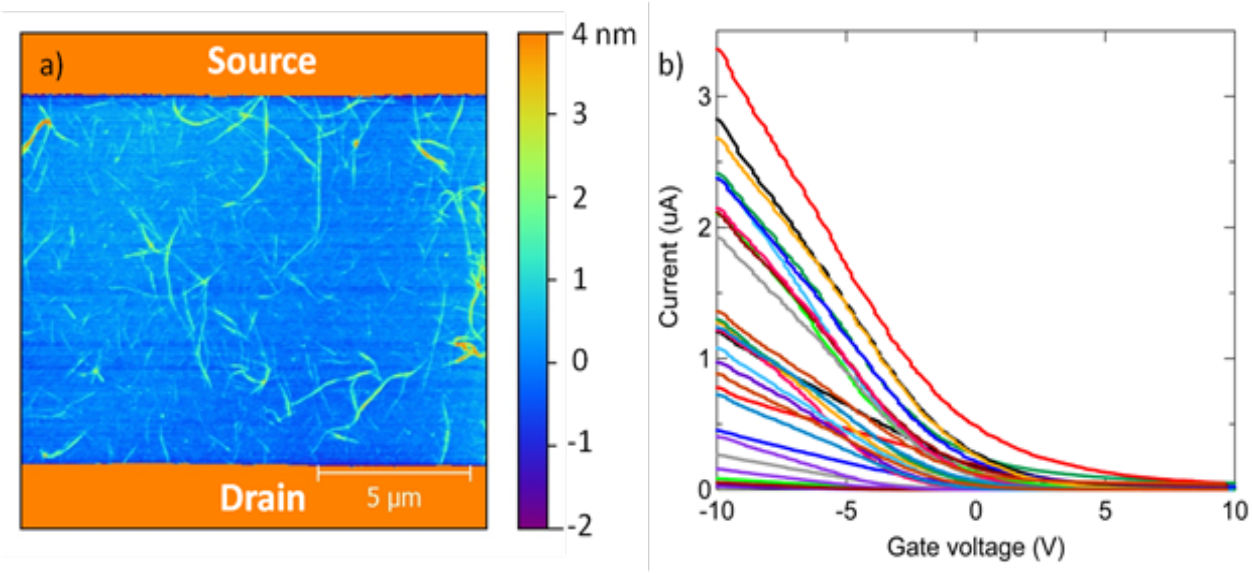

Figure 1: a) AFM image of a typical device showing a sparse nanotube network between electrodes. b) I(Vg) curves of a representative set of 28 devices from one of the sensor arrays, measured with V_{DS} = 100 mV.

As a first experiment, two compounds found in the sweat mixture, 9-decenoic acid and hexanoic acid, were tested separately in a background of clean air. As expected based on other studies [20], the sensors showed positive, albeit different, responses to both acids (Figures 2a & 2b) with response times on the order of ten seconds (Figure 2c). We hypothesize that the positive responses are due to deprotonation of the carboxylic acids within a nanoscale water layer bound to the DNA/NT. This is consistent with acid dissociation constants of 4.86 and 4.78 for hexanoic acid and 9-decenoic acid respectively. For 9-decenoic acid, the limit of detection is below 1.2ppm, the lowest concentration tested here.

Next, the standard sweat simulant, as defined in Table 2, was tested. In Figure 2d, the dynamic responses of seq2 and seq8 functionalized devices to this mixture are shown. In this trial, the humidity was fixed at 100%, i.e. the headspace was diluted by fully humidified clean air. This approach allowed concentrations up to the pure simulant headspace to be analyzed without varying the humidity, ensuring that the sensors' responses could only be a result of the VOCs as everything else in the vapor transported into the sensor chamber was identical. All four sequences showed positive responses to the sweat mixture, as would be expected given that the components are various acids. The current change varied between 1% and 4% at the highest concentration, depending on the DNA sequence used.

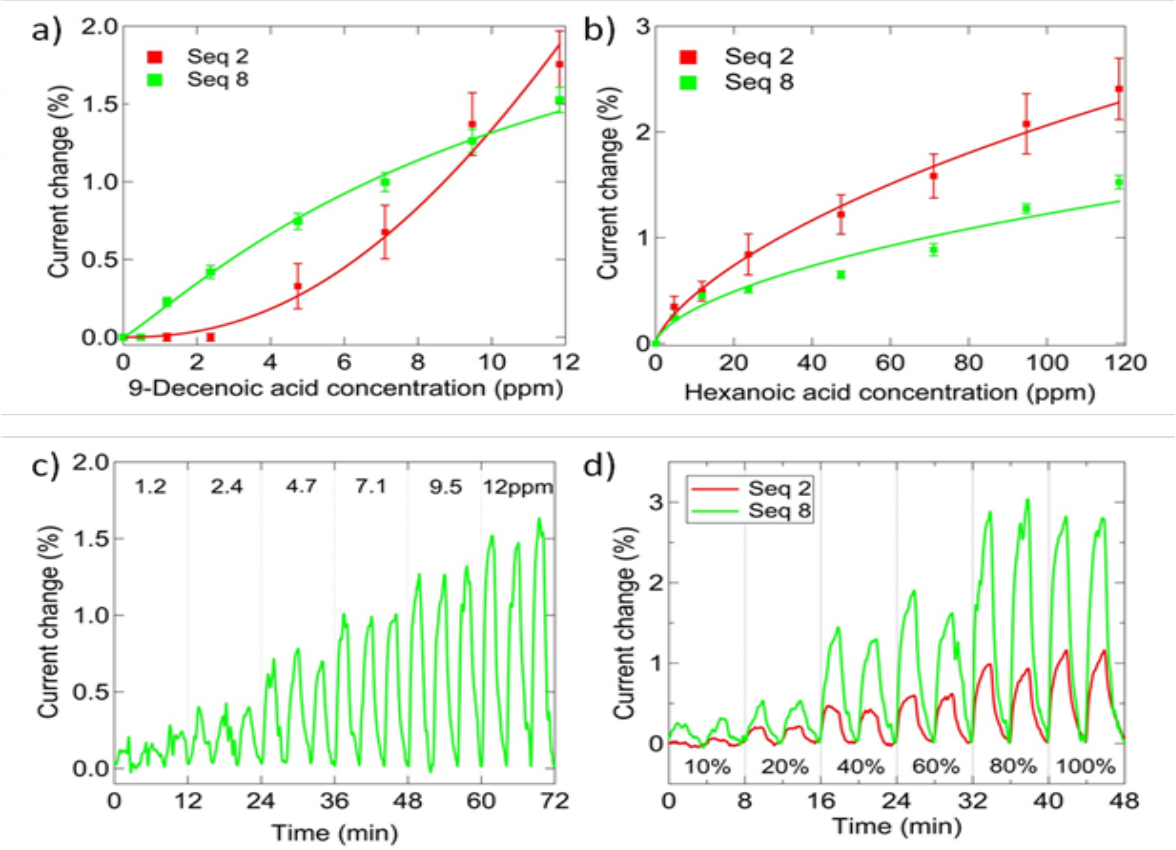

Figure 2: a) Current change as a function of 9-decenoic acid concentration for 2 different DNA sequences. b) Current change as a function of hexanoic acid concentration for 2 different DNA sequences. c) Dynamic data, averaged across 16 devices, used to get seq8 trace in a. The humidity level in panels a)-c) was 50%. d) Responses for seq2 and seq8 functionalized devices to the synthetic sweat mixture at increasing concentrations. Humidity was fully saturated for this trial.

As a first step toward demonstrating and quantifying the ability of DNA-NT devices to detect changes in the composition of the simulant mixture, sensors based on seq3 were exposed to the standard mixture and a mixture where the compound 3H3MH was omitted. The sensors were able to distinguish the two mixtures, with smaller responses observed for the 3H3MH-free solution (Figure 3a).

Next, a series of experiments was then carried out in which the concentration of a component in the mixture was varied between zero and 500% of the standard concentration. Two components were chosen for this, 3M2H and 9-decenoic acid. 3M2H was chosen because it has been shown to be a principal contributor to human underarm odor [24] and suggested as a uniquely human odorant [29]. Furthermore, 3M2H concentrations, as well as other axillary odorants, have been shown to vary across ethnicities [3]; the standard mixture, detailed in Table 1, contains a 3M2H concentration of 100 mg/L, a value chosen based on studies of Caucasian males' axillary sweat. Recent studies on a group of Japanese males found that the concentration of 3M2H in their axillary sweat is much lower, in the range 0-5 mg/L [3]. 9-decenoic acid was chosen as a second component to vary due to its lower concentration in the simulated sweat solution (and headspace), which would make changes in its concentration more challenging to detect.

The concentrations of components in the headspace of the mixtures were not measured directly. 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 standard solution, this yields a headspace concentration of 2 ppb for 3M2H and 0.02 ppb of 9-decenoic acid. Although it is known that real solution mixtures may show deviations from Raoult's law due to non-ideal mixing and chemical interactions between the constituents that change the components' effective vapor pressure, it is likely that the concentration of these VOCs in the headspace is extremely low (few ppb level or smaller). Moreover, the data presented below demonstrate that the DNA/NT sensor system is able to differentiate between mixtures where the amount of these compounds was changed by as little as 50% of the standard concentration. This implies that even this modest-sized DNA/NT array is capable of detecting changes of just a few ppb in one component of a 13-component vapor mixture.

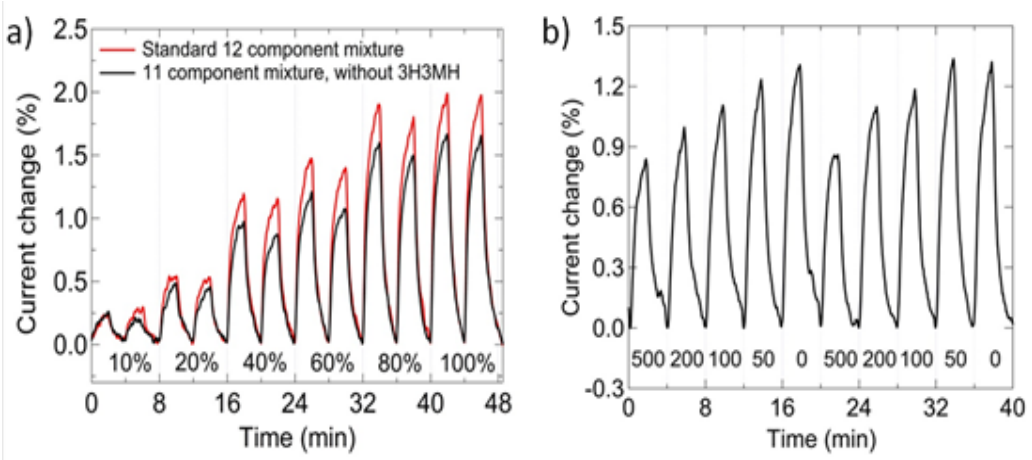

Figure 3: a) Dynamic responses from DNA/NT devices based on seq3 to vapors from sweat simulant mixtures differing only by the omission of one component, 3H3MH. Neither solution included 3M2H, which was obtained after this measurement was conducted. b) Dynamic responses from seq3 devices to 50% saturated vapor of 5 solutions of synthetic sweat with varying concentrations of 3M2H. Labels (0-500) refer to the percentage concentration of 3M2H in the solution responsible for each vapor pulse, referenced to the standard solution of Table 2. The set of measurements is repeated twice, and excellent reproducibility is observed.

Figure 3b shows typical time-dependent data for this set of experiments, in this case the averaged signal from 12 identical prepared DNA/NT sensors in an array of devices. Solutions contained 0, 50, 100, 200 or 500% of the standard concentration of 9-decenoic acid or 3M2H, with the concentrations of all other components held fixed. The devices were exposed to the 5 solutions sequentially (in each case, a vapor sample was passed across the sensors for 2 minutes, after which the chamber was flushed with clean air for two minutes), and the series of measurements was repeated twice. Figure 3b shows clear differentiation of the five solutions with varying 3M2H. Interestingly, the response magnitude decreases as the concentration of 3M2H is increased. While this may appear counterintuitive given that 3M2H is a carboxylic acid, it is possible that this occurs due to competitive binding between 3M2H and other molecules on the sensor surface, so that the presence of additional 3M2H prevents the binding of other acids that generate larger responses.

As shown in Figure 4, opposite effects were observed for each of the four DNA sequences upon increasing either the concentration of 9-decenoic acid or 3M2H in the solutions: the sensor responses increased for all four DNA sequences as 9-decenoic acid (circles) was increased but decreased with increased relative concentration of 3M2H (squares). In each plot, the response magnitude for the standard solution is normalized to 1, allowing for easier comparison between sequences. The trends are similar across all sequences, with additional 3M2H leading to a decreased response and additional 9-decenoic acid increasing the response for all sequences. However, the magnitude of the effect varies with DNA sequence: Seq2 and seq3 show the largest overall change in response magnitude, while seq4 is particularly sensitive to low concentrations of 9-decenoic acid and most insensitive to 3M2H.

We can claim that two solutions are distinguishable by an array of DNA/NT based on these four DNA sequences if the sensor response error bars do not overlap for at least one sequence. Using this definition, the set of 3M2H-varying solutions are all distinguishable from each other. The set of variable 9-decenoic acid solutions contains one pair (100% and 200% of the standard concentration) that is not significantly distinguishable based on the limited data set above. Also, if we consider the full set of 9 different solutions, another currently indistinguishable pair emerges (500% 9-decenoic and 0% 3M2H). However, we would expect that by increasing the number of devices or the number of different DNA sequences used, full distinguishability should be obtainable. Furthermore, statistical techniques such as principal component analysis could be employed to determine the linear combinations of the raw data that best distinguish the solutions.

In conclusion, we have demonstrated a viable technology for fast, cheap, all-electronic analysis of known odor profiles based on DNA/NT vapor sensors. Synthetic solutions were chosen to allow controlled composition changes to be made, and a set of very similar solutions was distinguished using arrays based on 4 different DNA sequences. The techniques used here could be scaled up to include tens or hundreds of DNA sequences being read out simultaneously, while remaining small and cheap enough to be incorporated into a handheld device. The chemical diversity of DNA suggests that such a set of sensors would have great analytical power, allowing odor-based chemical biometrics to advance from a research topic to truly impactful health and forensic applications.

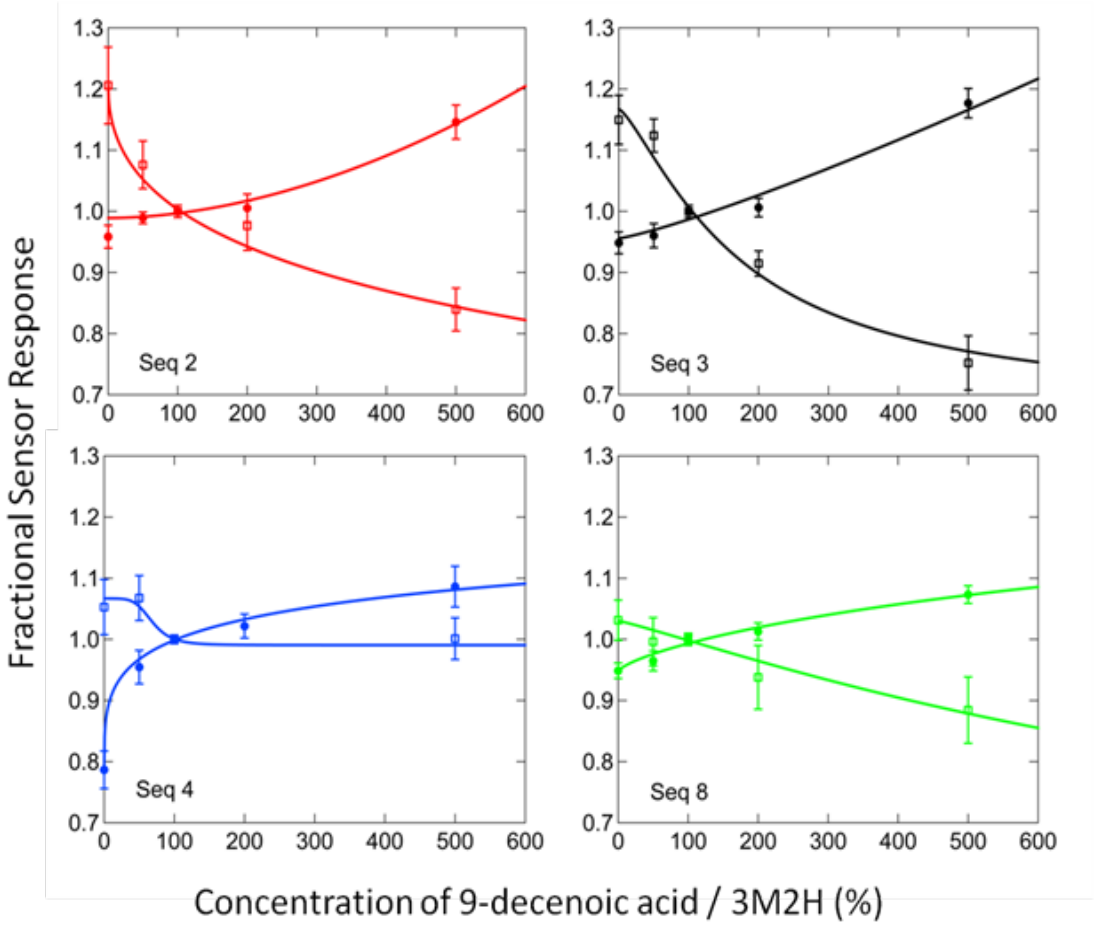

Figure 4: Normalized sensor responses for DNA/NT based on 4 different DNA sequences as the concentration of 9-decenoic acid (filled circles) and 3M2H (open squares) are varied. The concentration of 9-decenoic acid / 3M2H is varied between zero and 500% of the standard amount. The error bars reflect the spread of fractional sensor responses. Between 7 and 17 devices were used in each experiment.

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