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14. ABSTRACT
We investigated DNA oligomers as sorptive materials for miniature, solid-state electronic chemical sensors. We demonstrate successful integration of DNA with solid-state sensors using composites of DNA functionalized nanoparticles as chemiresistor devices. We investigated the scaling of chemiresistors down to nanometer dimensions. We demonstrate integration of many different sensing materials onto a single sensor chip. We demonstrate a path to combine a large number of DNA aptamers with nanoscale device arrays to achieve integrated, solid-state, sensor chips with specificity.

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Integration of Biological Specificity with Solid-State Devices for Selective Chemical Sensing

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I. Introduction & Overview

Chemical sensors have potential to be valuable tools to counter improvised explosive devices (IEDs) and defeat bomb-making networks. They can be used both for detection and identification of explosive materials, and made standoff by integration with land and aerial drones. In addition to searching for concealed explosives, one of the most powerful applications may be routine identification of chemicals in a non-intrusive way that helps find and track sources of raw materials used in bomb making. For this to be practical, it is necessary that sensors be low power, standalone instruments that can be widely deployed, rather than complex and cumbersome laboratory instrumentation. The concept that underscores our work is that chemical sensors can be made using nanofabrication methods and integrated with modern smart phones for low cost, widely deployable, non-intrusive tools that can be used to identify unknown substances and detect chemical signatures of hidden or unknown materials.

Current generations of stand-alone sensing materials can distinguish different classes of compounds and detect the presence of chemical signatures in a uniform background (air), but have trouble with unregulated environments and complex mixtures where there may be interferents. The trouble with mixtures originates from a lack of specificity and the limited number of materials used in current sensor designs. By contrast, analytical laboratory instrumentation often uses chromatography to separate components so that they can be detected with simple, non-selective detectors. The goals of miniaturized chemical sensors are to distinguish chemical targets in complex mixtures through selective and specific chemical interactions, without the need for chromatographic separations.

The chemical properties of many modern sensor materials, including both organic and inorganic polymers, can be described successfully using linear solvation energy relationships (LSER)[1]. LSER analysis decomposes a sensor material response into the fundamental chemical interactions including hydrogen bonding (H-bonding), dipolarity, polarizability, and dispersion. Selectivity for analytes originates from quantitative differences in these basic chemical interactions. The differing strengths of these chemical interactions can provide chemical selectivity sufficient to allow identification of single analytes in a uniform background, as well as simple mixtures through pattern recognition algorithms and principle component analysis. However, modern sensing materials generally do not include specific chemical interactions that can distinguish closely related compounds, or detect a particular target in a background of other vapors.

By design, LSER analysis of sensor materials quantifies fundamental chemical interactions, but does not take into account the specific shapes or geometric configurations of analytes. By contrast, the concept of specificity is commonplace in biochemistry and biomolecular engineering, where subtle changes in molecular structure can have dramatic effects on chemical interactions and biomolecular recognition. For example, the exact placement of a H-bond center in a molecule can determine whether that chemical interaction can be utilized for binding to a receptor.

Likewise, the exact placement of a side branch as small as a methyl can lead to steric interactions that cause binding differences of several orders of magnitude! The geometric stereochemistry of a molecule can effectively turn on and off various chemical interactions with different receptor sites, which yields molecular recognition and binding specificity. These specific effects are not captured in LSER analysis, which only considers the average chemical properties of a chemical compound.

Recent efforts in sensor research have investigated complex materials such as supramolecular compounds and molecular imprint polymers with the goals to design materials with enhanced specificity. On the other hand, the concept of specificity, including for detection of small molecules, is already well established in biomolecular science and engineering. In particular, DNA and RNA aptamers are a class of man-made receptors with a high degree of specificity that rivals proteins. DNA aptamers are relatively short sequences of nucleotides (A, T, G, C) that fold into complex 3D chemical structures with binding properties that can be made highly specific for chosen analytes. Aptamers are single-stranded DNA oligomers generally less than 100 base pairs, which have been engineered to bind to specific analytes through a laboratory procedure known as SELEX [2,3]. The number of unique single stranded DNA sequences scales as 4^N, where N is the length of the sequence. Thus, even modest values of N = 20 to 30, provide enormous degrees of freedom in the 3D structure of DNA oligomers. Complex 3D structures result from the ability of nucleotide bases to fold back on themselves to form A:T and G:C pairs, and complex stacking motifs that confer specific, hierarchical structures to DNA strands. Aptamers are distinguished from random DNA oligomers through the SELEX process, which seeks to discover specific sequences that bind strongly to given targets.

In the SELEX process, a very large library of ~ 10^{15} different random ssDNA sequences is exposed to analyte target(s) of interest, and only those sequences that bind to the target are retained, while the vast remainder are washed away. After several rounds of increasingly stringent selection criteria, the final sequences show highly specific binding to the desired analytes. The final DNA oligomers can be sequenced to know their identity. Generally, a number of different sequences are retained in the final pool, but they often show highly conserved regions that are believed responsible for actual binding to the target(s). During the SELEX process, one can also counter-select against other targets to improve specificity against known interferents. Once a single-stranded DNA (ssDNA) oligomer is identified and sequenced, it becomes an aptamer. The aptamer can be faithfully reproduced by DNA oligomer synthesis methods, using commercially available resources. The aptamers can also be modified to see how specific mutations affect binding to targets; some mutations have little effect on binding and some can enhance or degrade it. The general concept is that aptamers could be developed for any analytes of interest, including small molecules relevant to the detection of IEDs. For example, aptamers have been reported for TNT. Currently, aptamers are studied almost exclusively in the context of biology, but our work has been to investigate how to integrate aptamers and their binding specificity into solid-state electronic devices.

This work should be placed in the context of a broader scientific effort where the role of specificity in chemical sensing is unknown and there is a large knowledge gap between man-made chemical sensors and the biological function of olfaction.

II. Summary of Accomplishments

In this work, we initiated an investigation of DNA oligomers as materials for chemical sensors. The challenge has been to integrate DNA with solid-state devices for miniature, nanoscale sensors. Our work has proceeded along two independent paths: one has been to investigate DNA as a sensing material using standard chemical sensor designs, and the other has been to investigate nanoscale sensors using simpler, non-DNA sensing materials. These two paths reflect the two great potential advantages of DNA as a sensing material, which are enhanced specificity and unlimited diversity of receptor design. Our "big idea" is to integrate hundreds of different DNA sequences, each with some degree of specificity, into dense nanoscale arrays that can be made using nanofabrication methods. In this work, we did not specifically investigate aptamers because the number of aptamers that have been developed (and published) for small molecule targets is very limited, and the development of new aptamers would be itself a large effort that is outside the scope of the research project. Rather, we investigated the integration of DNA oligomers with solid-state devices and characterized DNA materials properties using LSER analysis.

The conclusions from our work are that it is possible to integrate DNA with solid-state sensors using networks of DNA functionalized nanoparticles as chemiresistor elements. In the biomolecular field, fluorescence is the most common technique to study chemical binding between targets and receptors. Our work with chemiresistor nanoparticle networks is the first to extend DNA sensing to electronic signaling. We find that DNA oligomers have similar sensing characteristics in terms of sensitivity and selectivity as carbon based polymer sensor materials. LSER analysis shows that DNA is distinguished as being a very strong hydrogen bond acceptor. Our work shows that non-Aptamer DNA oligomers are as good as other sensing materials that have been studied. We also find, not surprisingly, that DNA is particularly sensitive to moisture. The interference of water vapor has been a problem for most sorption based chemical sensors, and given the biological origins of DNA, it is not surprising that DNA covets water. In the present work, DNA oligomers were used in a dry state after casting and solution evaporation. Thus, our vapor sensing experiments were performed under a controlled relative humidity, with relatively dry conditions. Our conclusion is that DNA sensors should be investigated under wet conditions using either salted buffer solutions or a hydrogel environment. Proper buffer conditions will be particularly important for extensions of our work to DNA aptamers. The function of aptamers requires specific buffer solution environments that enable complex 3D structures from base pairing. Vapor sensing will require a mass transfer interface to move molecules from the gas phase into solution. Preliminary work not discussed in this report shows that we can extend analyte sensing from vapor to solution environments using our chemiresistor design integrated with microfluidic channels. We do not see any limitations for the integration of solid-state aptamer sensors with liquid flow cells.

We have also investigated the scaling of chemiresistors from the micron scale down to sensing volumes with nanometer dimensions. For these experiments, we used nanoparticles functionalized with small organic molecules, but the future extension to DNA is straightforward. We demonstrate that it is possible to maintain sensor function even when the conduction path is only 50 nm in length. We discovered that the sensing mechanism of vapor-induced swelling scales from micron size down to nanoscale devices. Both sensors give the same patterns of selectivity when testing similar sensing materials against the same group of vapor analytes. We demonstrate that it is possible to use dielectrophoresis to assemble nanoparticles with different sensing chemistry into adjacent nanoscale arrays and integrate many different sensors onto a single silicon chip. We conclude that it is possible to integrate hundreds of different sensing materials into large arrays of nanoscale devices, all integrated into a single chip. In the long run, the idea would be to combine a large number of aptamers with nanoscale device arrays to achieve integrated sensor chips with specificity.

Future work should investigate DNA aptamers using known aptamer/small molecule target pairs. Aptamers and DNA oligomers should be integrated with nanoscale sensor arrays and tested both in dry and solution environments. The successful demonstration of molecular recognition and binding specificity using aptamers will be transformative for chemical sensors research.

III. Results & Discussion

There have been three major investigations in this work: 1) investigations of the fundamental properties of ssDNA as a sensing material, 2) integration of biomolecular recognition elements with solid-state electronic devices, and 3) nanofabrication of nanoscale sensor arrays. Below, we provide a brief summary of the major results for each thrust. Further details are available in our publications listed at the end of the report. The last manuscript is currently under review and is available upon request. Portions of this work have also been presented at the SAAET review sessions.

A. Chemical properties of ssDNA as a sensing material

The chemical properties of DNA as a sensing material were investigated using the LSER analysis method. As introduced above, LSER is a quantitative method to analyze sensor data to understand the fundamental components of the non-covalent bonding interactions between a sensor material and analytes [1]. The sensor material – analyte interaction is divided into complimentary, reciprocal properties of sensing materials and analytes through several parameters. Sensing materials and analytes each have a set of parameters that reflect the fundamental chemical interactions promoted by the particular analyte or sensing material. For example, the sensing material will have a capability to both give and receive H-bonds; likewise an analyte will also have the capability to give and receive H-bonds. If the two properties are complimentary such that the sensing material is an effective H-bond acceptor and the analyte is an effective H-bond donor (or the opposite), then there will be a strong

chemical interaction between the analyte and sensing material. Mathematically, the parameters of the sensing material and analytes are multiplied together so that large multiplicative products make a large contribution to the binding interaction. Conversely, if the two chemical interactions were miss-matched, then there would be a small product and weak contribution to binding. Besides H-bonding, LSER also considers polarizability, dipolarity, and dispersion. All together there are 5 pairs of complimentary parameters; each has a parameter for the analyte and a parameter for the sensing material. Parameters for a large number of analytes and a moderate number of sensing materials (primarily polymers) have been tabulated. Thus, it is possible to predict the binding interactions of a sensing material for many analytes if the LSER parameters of both the material and analyte are known. Moreover, the magnitudes of the LSER parameters reveal the relative significance of the fundamental non-covalent chemical interactions for different sensing materials. In this way, the chemical properties of a sensing material can be understood from the fundamental chemistry.

Note that by definition, LSER does not include any type of specificity in the chemical interactions. There are no terms that describe steric interactions, binding pockets, or geometric shape properties of either the sensing material or the analytes. Rather, LSER describes non-specific covalent interactions that are associated with the solubility of an analyte into a host sensing material. However, these interactions can be selective, and do lead to significant differences for different analyte/sensing material pairs. When complimentary properties of a material and an analyte match, there can be a significant enhancement of chemical interactions compared to other analytes, so there is selectivity. There is a subtle, but important difference between selectivity and specificity. The former is achievable with modern sensing materials, but the latter has not been studied in depth.

The purpose of our LSER studies of DNA oligomers are two-fold: 1) LSER analysis reveals the fundamental chemical interactions that help to understand DNA as a sensing material, and 2) LSER characterization provides a baseline to describe DNA as a selective sensing material so that we can later study specificity. The first point has been addressed through investigation of the composition dependence of DNA on chemical interactions. DNA is made up of sequences of 4 nucleotides as well as a phosphate backbone. There is also a hydration shell composed of strongly bound water and counter ions. We have studied if there are observable differences in the chemical properties of DNA for different A, T, G, C content. The second point has to do with specificity and the particular sequence of nucleotides. Aptamers are unique ssDNA sequences that dynamically fold into complex 3D structures where binding specificity originates from the structure and not simply the chemical composition of the DNA. Note that this is significantly different than all previous sensor materials, whose properties derive from composition and not structure. (There have been related efforts using complex cavity shaped molecules, but we are not aware of any verifiable evidence to support a claim of molecular recognition and specificity). Also, note that LSER does not consider binding pockets or specific interactions that might arise from 3D structure.

Our working hypothesis is that to study specificity, we must distinguish geometric structure effects from non-specific covalent chemical interactions that promote selective binding. Thus, LSER provides a quantitative means to characterize selective, but non-specific chemical interactions. To prove specificity, we must show that an aptamer has binding characteristics that are statistically above the baseline of non-specific chemical binding interactions defined by LSER analysis. We can use mutated aptamers with the same chemical composition (and hence the same LSER properties) to disentangle composition from structure. In the long run, this is how we intend to identify, study, and prove specificity in chemical sensing. Note that some materials have been claimed to have specificity only to be later disproven by LSER analysis [4]. Definitive demonstration of specificity (outside the context of biology) would be a breakthrough for chemical sensing.

Table 1
DNA sequences.

Name	Sequence (5'–3')
$(dA)_{25}$	(25 repeating units of dAMP)
$(dT)_{25}$	(25 repeating units of dTMP)
$(dC)_{25}$	(25 repeating units of dCMP)
$(dA)_{10}$	(10 repeating units of dAMP)
$(dA)_{50}$	(50 repeating units of dAMP)
27A	ACC TGG GGG AGT ATT GCG GAG GAA GGT
27L	AAG GAG AGG GAT GAT GGT TGT GCG CGC
24A	AAA AGG GGA AAA AAA ACC CCT TTT
24L	GAA TTA ACA AAC CAG ATA ACG ATG

In this work, we have investigated the LSER properties of 7 different ssDNA sequences, including different length homo-polymers of deoxyadenosine monophosphate, deoxythymidine monophosphate, and deoxycytidine monophosphate, as well as 4 other mixed sequences (Table 1). One of the sequences studied is the same sequence as the aptamer for ATP (adenosine triphosphate). It was chosen for study because it is a relatively simple aptamer, well studied, and known to fold into a complex 3D structure. However, we did not test the aptamer against the ATP target, because the latter requires a liquid solution environment and our studies have been for vapor analytes. Moreover, the aptamer was designed for a particular liquid solution environment, which is likely required for its 3D structure. In general, aptamers have only been studied in solution environments, and none have been developed for sensing of vapor targets. Thus, our interest in the ATP aptamer is only for the different chemical structure compared to the ssDNA homo-polymers. It is not known if the ATP aptamer maintains a folded 3D structure in a dry, condensed film. In very recent work, we have extended our chemical sensing studies to microfluidic devices using solution environments for ssDNA experiments, but these are beyond the scope of this grant.

LSER analysis of our ssDNA materials requires a set of analytes with known LSER parameters and data for the equilibrium binding of the analytes with our sensing materials. From these data, the LSER parameters of our ssDNA sequences can be determined by multiple linear regression. We used a quartz crystal microbalance (QCM) to measure quantitative equilibrium binding constants for different analytes tested against the seven different DNA materials (Table 1). The DNA materials were cast as thin films (< 100 nm) on QCM crystals and the equilibrium mass uptake was measured for each analyte under dry conditions. A few polymers were also studied as reference materials with known LSER properties. Based on prior LSER studies of polymers, we analyzed 20 different analytes for each ssDNA material. The analytes

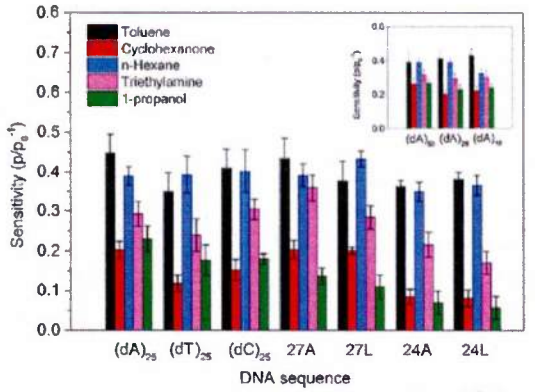

Figure 1. Average sensitivity on a p/p_0 scale of seven DNA coated QCM sensors responding to a group of 5 volatile compounds. The inset shows the response for sequences of the same composition, but with different lengths.

were chosen to have a broad set of chemical properties to aid in the accurate determination of all the LSER parameters; 20 analytes are deemed sufficient for accurate determination of new parameters. Note that once parameters are determined for our ssDNA materials, it is possible to predict the binding interactions for more than 1000 vapor analytes, limited only by the number of analytes that have been characterized by the LSER method.

The sorption experiments were very successful; each ssDNA material showed fast, reversible, and sensitive uptake of the vapor analytes. The analytes were studied for partial pressures near $p/p_o = 0.01$, where p_o is the saturation pressure. We observed strong selectivity for different analytes, and significant differences between ssDNA of different composition. Figure 1 shows a summary of data for our 7 DNA sequences and a subset of 5 vapors. The y-axis is a measure of the sensitivity of the sensing material toward the analytes. The differences of the response to different analytes (i.e. selectivity) are similar in magnitude to previous publications using synthetic polymers. The data show significant differences in the response properties of different DNA sequences. For example, compare the responses of 27A and 24A. The different response sensitivities are well above error bars for repeatability, and the relative ratios are very different. These material differences can be used for pattern recognition analysis of vapor mixtures. The data demonstrate the power of DNA as a sensing material. For other materials like synthetic polymers, it is necessary to select a different material to vary response selectivity, but for DNA we can change the composition by changing the sequence.

We also analyzed ssDNA responses for possible structure effects. For example, compare the responses of 27A and 27L; these oligomers differ in the exact sequence of nucleotides, but have the same overall composition (same A,T,G,C content). The 27A sequence is the ATP aptamer, known to have a complex 3D structure in buffer solutions (but uncharacterized in a dry, thin film state). There are small differences in the responses (especially for triethylamine), but our overall analysis concludes that within the repeatability of the experiment, we cannot identify any definitive structure effects. The results are not surprising considering that the ATP aptamer was not in its native form, and was not tested against its known target (ATP).

LSER analysis shows that DNA has similar chemical properties as synthetic polymers with a carbon backbone, but that DNA has much stronger hydrogen bond basicity. Figure 2 summarizes the LSER parameters r, s, a, b, & l that were determined in this work. The strong H-bond basicity is evident from the large 'a' parameter, which is

comparable to the upper end of the range of known values, exemplified by siloxane polymers with amine and carbonyl groups. These data define the fundamental interactions of ssDNA as a sensing material and provide a baseline for the study of aptamers. Overall, our conclusions are that we can use LSER analysis to study specificity and the effects of structure on analyte binding. However, given that existing aptamers for small molecules have only been developed for liquid environments, we recommend that future studies be performed in solution, under conditions that promote 3D structures of aptamers, and that aptamers be tested against known targets. Once specificity is quantified and understood, it should be possible to design new aptamers for new targets relevant to counter-IED. For example, aptamers have already been developed for TNT and could be developed for other compounds used in explosives.

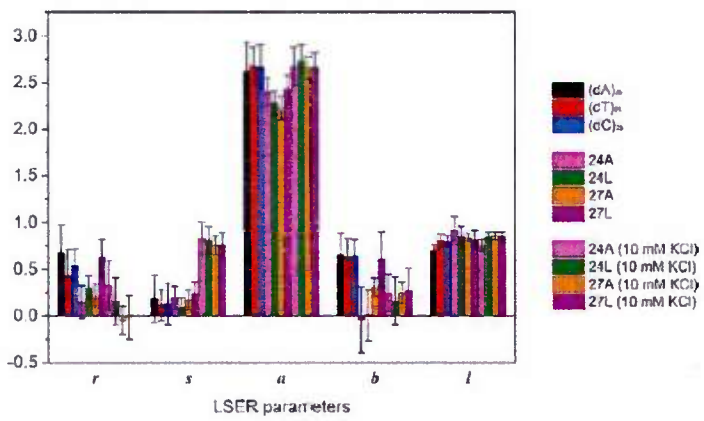

Figure 2. Bar chart of derived LSER parameters for the 7 ssDNA sequences studied in this work. Four sequences were also studied by preparing in a different salting environment (10 mM KCl). Note the significant magnitude of the 'a' parameter.

B. Integration of biomolecular recognition elements with solid-state electronic devices

The vast majority of sensing research with biomolecules is carried out in solution using fluorescence based detection of molecules that are tagged with fluorescent probes, and measured using laboratory analytical techniques. The objectives of this work were to investigate electronic devices that could be made for sensitive, low cost, portable sensors without the need for laboratory instruments such as laser sources, spectrometers, chromatography, etc. We investigated chemiresistor devices as an approach to integrate biological recognition elements with solid-state electronic devices. As will be demonstrated in the next section (section C) chemiresistors are sensitive transducers that can be scaled to nanometer dimensions and still provide robust signals.

Chemiresistors utilize a sensing material that changes resistance upon exposure to vapor analytes. The devices are simple, sensitive, robust, easily fabricated, and scalable to the nanoscale. In particular, nanoparticle composites have proven to be very effective chemical sensors. The concept is to lay down a layer of organically functionalized metallic nanoparticles (typically gold) between two interdigitated electrodes. Organic molecules are bound to Au nanoparticles through strong thiol or amine bonding that displaces weaker bound species used in the preparation of the nanoparticles. The nanoparticles are typically in the range of 2-100 nm diameter and

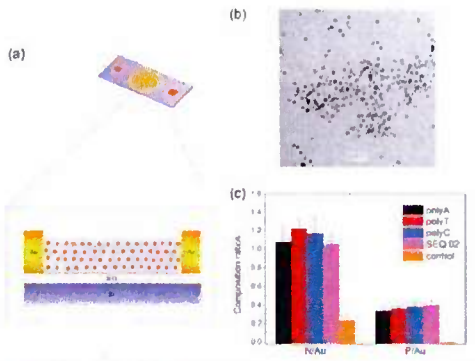

Figure 3. (a) Schematic of a chemiresistor device using a circular electrode design, (b) TEM image of 10 nm nanoparticles used to make sensors, (c) composition ratios of N/Au and P/Au measured by XPS to confirm DNA assembly on Au Nanoparticles.

the organic layer thickness around the particles are less than 2-3 nm thick. Figure 3 shows a schematic of the concept using a circular design for the inter-digitated electrodes with an overall size of several mm^2, and an electrode spacing of 5-20 um.

The metallic particles provide conductivity to the medium between electrodes, and the organic component provides a medium for sorption of vapors. Because the organic medium is not a conductor, electrical charge transport between electrodes must occur by an electron hopping mechanism where electrons tunnel through the organic medium between particles. The probability of electron tunneling scales exponentially with the distance between particles. Thus, when a nanocomposite of functionalized nanoparticles is exposed to analyte vapors, vapors will partition between the vapor phase and the organic phase. Thermodynamics dictates that vapor analytes will accumulate in the organic phase and this leads to swelling of the composite film and small increases in the spacing between adjacent nanoparticles. Even a small degree of swelling is transduced as an increase in the electrical resistance of the device, so that analytes can reliably be detected at levels down to 0.001 of the saturation pressure. Using different organic phases leads to selective responses that can be used in pattern recognition for analyte identification. Selectivity derives from the same non-covalent interactions that are described by LSER.

In this work, we investigated nanoparticles functionalized with ssDNA as the organic phase for analyte detection. In very recent work, we have extended sensing experiments to liquid solutions using microfluidic techniques, but the work described here is confined to experiments with vapor analytes. We developed methods to functionalize Au nanoparticles with 25-mer ssDNA sequences, including poly A, poly T, poly C, and mixed sequences. Fluorescence techniques were used to verify successful attachment of ssDNA to gold nanoparticles (AuNPs). The DNA functionalized nanoparticles were incorporated into sensor electrodes using a simple drop-cast method followed by drying. The condensed DNA films were characterized using electron microscopy, spectroscopic ellipsometry, and x-ray photoelectron spectroscopy. As shown in Figure 3, the presence of Phosphorus is one indicator of the successful incorporation of DNA into our solid-state sensing devices. The DNA sensors were tested against several analytes under different relative humidity conditions using a custom built flow cell. Electrical instrumentation was used to measure electrical resistance during pulsing and purging of analytes from the flow streams using pure nitrogen as a carrier gas. The main variables were the partial pressure of the analytes and the relative humidity, which was varied from 0% to 99%. The analytes tested included ethanol, methanol, hexane, toluene, and DMMP.

The DNA sensors responded to each vapor analyte with different sensitivity, and there were different response patterns for the 4 ssDNA sequences. At low relative humidity, the DNA sensors were qualitatively similar to organothiol functionalized Au NP sensors previously reported in the literature. Figure 4 shows an example of real time responses for a few different DNA sequences responding to increasing vapor concentrations of hexane. The major advantage of ssDNA is that there are virtually unlimited numbers of unique sequences for pattern recognition, whereas the number of organothiol compounds is limited. However, the DNA sensors showed a very strong response towards water vapor, which is a disadvantage for sensing in uncontrolled relative humidity environments. Sensitivity to water is a problem for most sensing materials, but was particularly acute for DNA. The affinity of DNA for water is not surprising given the biological origins of DNA. At higher humidity (40% to 100%), sensors behave increasingly like a polyelectrolyte with ionic conduction contributions to the electrical response. Overall, the chemiresistor experiments were successful and demonstrated that ssDNA has qualitatively similar sensing properties as other nanoparticle functionalization chemistries, with the caveat that DNA responds more strongly than most to water vapor.

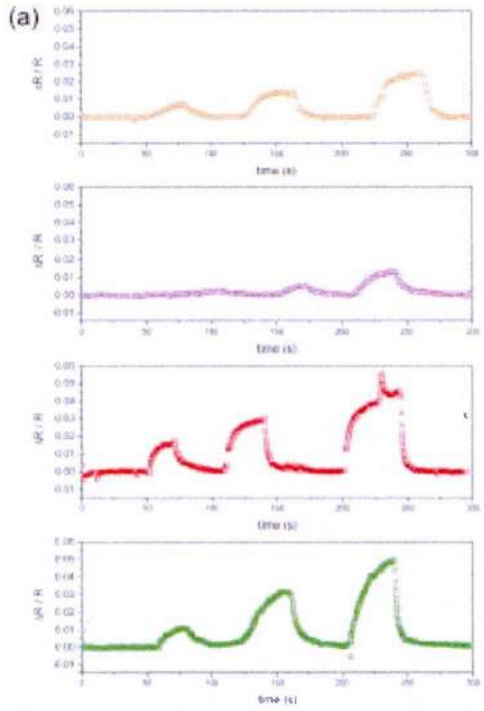

Figure 4. Real time sensor responses for four types of DNA functionalized gold nanoparticles responding to increasing concentrations of hexane. From top to bottom the sequences are: polyA, polyT, polyC, and /ThioMC6-Db/TTT TTA CTC CTT TTC CTC CTC TTT T.

Our major conclusions from this work are that ssDNA, including aptamers, can be successfully integrated into solid-state electronic devices using nanoparticle based chemiresistors. In the present work, we have investigated ssDNA oligomers not specifically targeted against particular analytes, as would be done using aptamers. However, the results demonstrate that it should be possible to make devices using ssDNA aptamers with enhanced specificity towards their target analytes. Such selective sensing would be a breakthrough for chemical sensors research. On the other hand, we also conclude that ssDNA, and by extension aptamers, are not suited to environments where the relative humidity is not well controlled. Sensor response to changes of humidity will exceed the response toward target analytes. The humidity problem is general to most sensing materials because variations in water vapor concentration are much larger than analyte concentration and will dominate sensor response even for materials that respond weakly to water vapor. We conclude that DNA aptamers should be investigated in salted, buffered, liquid solutions.

The use of a liquid environment solves several problems. The constant water background eliminates water as an interference, and the salted, buffer conditions will promote the complex 3D structures of aptamers that trigger highly specific binding to targets. We still believe aptamer sensors will be effective for vapor sensing, but it will require a mass transfer interface between air and a liquid sensing cell. In very recent work not described here, we have discovered that chemiresistors work well in liquid solutions. We foresee a future design where vapor analytes are transferred from air into a liquid or hydrogel layer that passes over a bank of aptamer functionalized sensor arrays.

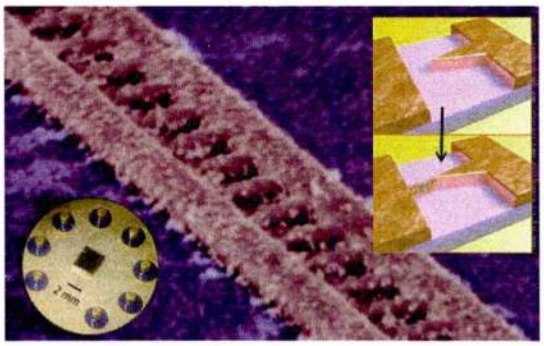

Figure 5. A multi-tip nanoscale chemiresistor is shown from a perspective view. Top: inset shows a device schematic, bottom: inset shows a sensor chip mounted to a TC package.

C. Nanofabrication of nanoscale sensor arrays

The above discussion has highlighted our work to characterize DNA as a sensing material with a vision toward future quantitative studies of specificity, and to integrate DNA into solid-state electronic devices that can be made using nanofabrication. Here, we highlight our work on nanofabrication and demonstrate how we could integrate sensors into large arrays with different materials all integrated onto a single sensor chip. Although not discussed here, this work is particularly relevant to theories of chemical sensing that argue large numbers of sensors are needed for effective algorithms that can distinguish components in mixtures and variable backgrounds [5].

Chemiresistor sensors based on Au NPs are generally designed to have thin films cast over micron scale electrodes, occupying mm^2 areas on a sensor (Figure 3). The advantages of such an approach are that sensor response is averaged over a large interfacial area and many conduction paths to give a robust signal with good S/N properties. However, the disadvantages of standard designs are that continuous conduction paths require relatively thick films of NPs, and integration of many sensors is limited by the large size of each sensor. Sensor response speed is limited by diffusion of analytes into the AuNP/organic composite layers, and the response rate scales unfavorably with thickness. Hence, most sensors have response and recovery rates that take 10's of seconds or longer, which can be much longer than the rate of change of the input chemical signals. Thinner films can be faster, but the thickness is limited by the need for uniform, continuous films to span across the electrodes.

Nanoscale devices have significant advantages over micron scale devices with only a small penalty of S/N. We have investigated the scaling of chemiresistors down to the nanoscale and we find significant enhancement of the response and recover speed. Figure 5 shows a schematic of a nanoscale chemiresistor, where a small number of Au NPs span between electrodes placed only 50 nm apart (top inset). The sensing volume

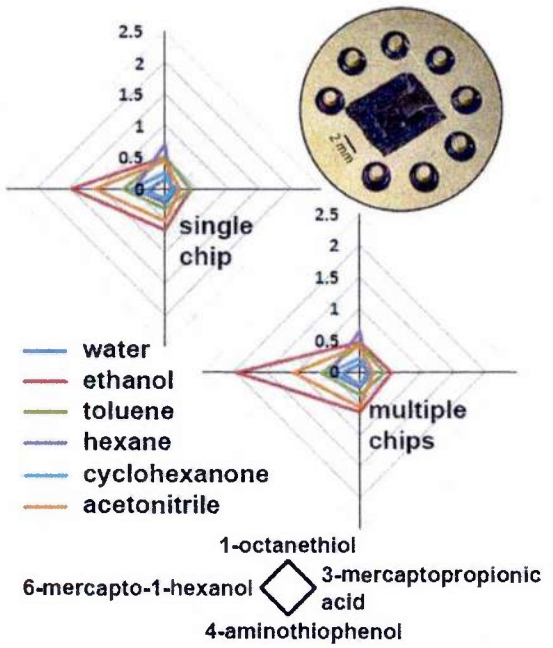

Figure 6. A single chip sensor with 4 integrated organothiol functionalized nanoscale chemiresistors is compared with 4 separate chips. The radar plots are a relative measure of sensitivity towards the 6 analytes.

is very small, with a height of approximately 50 nm, a lateral width approximately 50 nm and a length of 50 nm, for a sensing volume near 1.25×10^{-4} um^3 (per tip). We estimate that less than 50 nanoparticles make up the path between electrodes for each tip in the array. Arrays of 10, 100, and 1000 tips were investigated. The nanoscale devices respond and recover with time scales much less than one second. Most surprising is that the sensing mechanism based on bulk sorption and swelling of nanoparticle layers is maintained from the micron scale all the way to the nanoscale. One might expect that at some very small length-scale, the mechanism for sensing would change, but this was not observed here.

We investigated a series of organothiol functionalized AuNPs as sensing materials and compared the selectivity of the responses between micron-scale and nanoscale devices. We found that the selective response characteristics of the organothiols were maintained despite the size reduction by 7 orders of magnitude. The S/N was degraded by only a factor of 4, and could be improved.

Besides the speed enhancement, one of the primary advantages of nanoscale sensors is the ability to integrate many devices onto a single sensor chip. In order to integrate many different sensing materials onto the same chip, it is necessary to spatially separate the different sensing materials. For non-integrated chips, this is trivial, but package size and complexity scales unfavorably with the number of sensor chips. On the other hand, for nanosensors it is easy to design a compact layout and it may be possible to integrate communications and signal conditioning onto the same chip, but spatially separating the many different sensing areas is nontrivial.

In this work, we investigated dielectrophoresis as a means to assemble different sensing materials into adjacent devices on the same chip. During dielectrophoresis, a high frequency AC signal is applied to selected devices to attract nanoparticles to bridge between electrodes. We discovered that only devices with applied AC voltage lead to assembly. Thus, it is possible to sequentially select different devices and expose them to nanoparticles functionalized with different organic layers. In this way, it is possible to build a sensor with many different sensing regions, each with a different chemical selectivity, onto a single chip. By connecting multiple devices to the power source at the same time, it will be possible to add built-in redundancy and to improve the S/N

through independent sampling. Figure 6 shows an example of a single chip sensor compared with 4 separate sensors. The latter each have only one type of sensing material. The radar plots show that the sensor response patterns are similar for the two very different sensor designs. In future work, it will be desirable to investigate nanoscale sensors with nanoparticles functionalized by aptamers, and the single chip design concept could be scaled to have 100s of independent devices operating at the same time. In very recent work, we have demonstrated that these nanoscale sensors also work in liquid solution environments.

IV. Publications from this work

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